A prospective study of the association between depression and inflammation in type 2 diabetes

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A Prospective Study of the Association Between Depression and Inflammation in Type 2 Diabetes

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Abstract

There is a bidirectional association between type 2 diabetes mellitus and depression. People with type 2 diabetes mellitus and comorbid depression have an increased risk of premature mortality. Type 2 diabetes mellitus has an underlying inflammatory basis, and there may be related underlying inflammatory process in depression. However, there is little knowledge of how systemic inflammation is involved in this depression-diabetes link. This thesis examines whether there is a correlation between concentrations of 12 inflammatory markers and depressive symptoms in newly diagnosed type 2 diabetes mellitus. The primary hypothesis was that those with depression are more likely to have higher concentrations of inflammatory markers compared to those that are not depressed, adjusting for confounding (including obesity). The design was a prospective cohort derived from the South London Diabetes Cohort (n = 1790). Patients were recruited < 6 months post diagnosis of type 2 diabetes mellitus and followed-up at 12 months. Depressive symptoms were assessed using the Patient Health Questionnaire-9 and inflammation was measured using standard hospital assays and a biochip immunoassay. Multiple linear regression was used to adjust for covariates and factor analysis was used to identify underlying latent factors to explain the observed association between inflammatory markers and depressive symptoms. The findings were as follows; firstly, there was a cross-sectional association between depression and five inflammatory markers (C-reactive protein (CRP), interleukin-1 receptor antagonist, monocyte chemotactic protein-1, white blood cell count, and triglycerides). Secondly, a composite measure of inflammation including these five markers was more closely correlated with depression score than any individual marker. Thirdly, depression was associated with higher concentration of CRP at 12 months. Finally, baseline CRP was not significantly associated with increased risk for new cases of depression. These findings may help explain the poorer prognosis of those with type 2 diabetes mellitus and comorbid depression.

Statement of Work

The work comprising this PhD was carried out on the South London Diabetes Cohort (SOUL-D), a cohort of 1790 newly diagnosed type 2 diabetes mellitus patients. This study has been running since 2008 and is due to finish in September 2014. I took part in recruitment for the cohort and also follow-up appointments. Over the 3 years of my PhD I conducted interviews with approximately one quarter of the study population. I was trained in phlebotomy so that I could take blood samples from the patients when necessary. In addition, I collected data from GP records for several hundred patients who were not able to attend appointments. I worked at the pathology lab observing and assisting with the biochemical assays and also selecting aliquots for analysis. I had considerable input in the organising of storage conditions for the aliquots and in deciding which biomarkers to study. I had to organise the datasets at each time-point so that they could be analysed, this involved reorganising and recoding tens of thousands of data points. On commencing my PhD I observed that we were not collecting data about comorbid conditions for the patients in the cohort, data which I thought would be essential to have as it could be a major confounder for my research questions. Therefore, I created a supplemental page to be added to the 24-month follow-up data collection schedules so that we could retrospectively collect data on all long-term comorbid conditions suffered by patients during the study – unfortunately this data was not available for use in my PhD thesis. Although ethical approval had already been received for the work comprising this PhD, I did get experience in doing an ethics application and planning a small proof of concept study. Towards the end of my PhD I took on additional responsibilities such as being involved in the training of several new data collectors, supervising a BSc student, publishing data in academic journals and presenting findings at international conferences. I also contributed to weekly research management, collaborated with and covered colleagues on leave and shared workloads.

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Glossary of Abbreviations

ACE - angiotensin-converting-enzyme

ACTH - adrenocorticotropic hormone

ADA - American Diabetes Association

AGEs - advanced glycation endproducts

ANCOVA - analysis of covariance

ANOVA - analysis of variance

BDNF - brain-derived neurotrophic factor

CBT - cognitive behavioural therapy

CNS - central nervous system

COX-1 - cyclooxygenase-1

COX-2 - cyclooxygenase-2

CRH - corticotrophin releasing hormone

CRP - C-reactive protein

CSIF - cytokine synthesis inhibition factor (murine IL-10)

CT - computerised tomography

CVA - cerebrovascular accident

CVD - cardiovascular disease

C1 - Cytokine 1

C2 - Cytokine 2

C3 - Cytokine 3

DCCT - The Diabetes Control and Complications Trial

Depression - Major Depressive Disorder

DPP-4 - dipeptidyl peptidase-4

DSM IV - The Diagnostic and Statistical Manual for Mental Disorders IV

DSM-V - The Diagnostic and Statistical Manual for Mental Disorders V

EECT - electroconvulsive therapy

FBC - full blood count

FFA - free fatty acid

GABA - gamma-aminobutyric acid

GLP-1 - glucagon-like peptide-1

GP - general practitioner

GR - glucocorticoid receptor

GTT - glucose tolerance test

HADS - Hospital Anxiety and Depression Scale

HbA_{1c} - glycated haemoglobin

HDL - high density lipoprotein

HPA - hypothalamic-pituitary-adrenal

hs-CRP - high sensitivity C-reactive protein (assay)

ICD-10 - International Statistical Classification of Disease and Related Health Problems criteria

IFCC - International Federation of Clinical Chemistry

IFN-α - interferon-alpha

IFN-β - interferon-beta

IFN-γ - interferon-gamma

IGT - impaired glucose tolerance

IL-1 - interleukin-1

IL-1RA - interleukin-1 receptor antagonist

IL-4 - interleukin-4

IL-6 - interleukin-6

IL-10 - interleukin-10

IPT - interpersonal therapy

IQR - interquartile range

LDL - low density lipoprotein

MCP-1 - monocyte chemotactic protein-1

MAOI - monoamine oxidase inhibitors

MAO - monoamine oxidase

MRI - magnetic resonance imaging

NDMA - glutamate N-methyl-D-aspartate

NET - norepinephrine transporter

NF-κβ - nuclear factor kappa-light-chain-enhancer of activated B cells

NICE - The National Institute for Health and Clinical Excellence

NHS - National Health Service

NK - Natural Killer cells

PAD - peripheral artery disease

PET - positive emission tomography

PHQ-9 - Patient Health Questionnaire - 9

RCTs - randomised control trials

r_s - Spearman's ranked correlation coefficient

SCAN 2.1 - Schedules for Assessment in Neuropsychiatry

SERT - serotonin transporter

SD - standard deviation

SNRIs - serotonin norepinephrine reuptake inhibitors

SSRIs - selective serotonin reuptake inhibitors

TCAs - tricyclic antidepressants

TGL - triglycerides

Th - T helper

TNF-α - tumour necrosis factor alpha

UK - United Kingdom of Great Britain and Northern Ireland

US - United States of America

VEGF - Vascular endothelial growth factor

WBC - white blood cell count

WHO - World Health Organisation

χ^2 - Chi-squared

5-HT - serotonin

5-HTT - serotonin transporter

Chapter 1: Introduction

Synopsis

The overall objective of this thesis is to examine the association between inflammation and depression in a newly diagnosed type 2 diabetes mellitus population. The aim of this chapter is to explain why this population was chosen for studying the association between depression and inflammation in chronic illness. The concepts of depression and type 2 diabetes mellitus are discussed. Their epidemiology, causes (aetiology) and consequences (prognosis) as well as the management for both conditions (treatments) are discussed. This is followed by an overview of the evidence for common biological and psychological aspects of the two conditions, drawing attention to the limitations of the current evidence and giving context to the original contribution to knowledge of this thesis.

Depression Overview

Clinical Characteristics

The Diagnostic and Statistical Manual for Mental Disorders-IV (DSM-IV) precedes the current DSM-V and was in operation when this thesis was conducted. It defines Major Depressive Disorder (depression) as a continuous depressed mood or loss of interest/pleasure in daily activities that persists for at least 2 weeks (APA, 2000). Although there are small differences in the classification of depression by the International Statistical Classification of Disease and Related Health Problems criteria (ICD-10), the two systems of classification are broadly similar (Andrews et al., 1999). The typical duration of a depressive episode is between 3 to 4 months (Spijker et al., 2002; Kessler et al., 2003; Eaton et al., 2008), but at least a fifth of depressive episodes persist for 2 years or longer (Spijker et al., 2002).

Assessment

Depression is diagnosed by the presence of at least five symptoms which cause significant distress or functional impairment and are present almost every day for at least 2 weeks, based on DSM-IV (APA, 2000). A diagnosis is made from a clinical interview, and for research studies a semi-structured interview schedule such as the Schedules for Assessment in Neuropsychiatry (SCAN 2.1) may be used (Wing et al., 1998).

Although using a clinical interview is the most valid method available for identifying depression, it is not always practical for psychological research (as the assessments are lengthy and require extensive training). Consequently, most epidemiological research that has been carried out on depression relies on self-report instruments (Engum, 2007; Maraldi et al., 2007; Cooper et al., 2010) such as the Patient Health Questionnaire-9 (PHQ-9) (Kroenke et al., 2001) or the

Hospital Anxiety and Depression Scale (HADS) for anxiety and depression (Zigmond and Snaith, 1983). These generate a measure of depressive symptoms and probable depression cases rather than a diagnosis of clinical depression.

Epidemiology

Depression affects over 340 million individuals worldwide (Greden, 2001), at least 10-20% of individuals in high-income western countries (Bromet et al., 2011), 7.6% of males and 11.8% of females in the United Kingdom (UK) (McManus et al., 2009). In a clinical setting exact numbers are hard to estimate because many individuals with depression do not report symptoms to healthcare professionals (Oliver et al., 2005). Help-seeking patterns in males are consistently lower than in females (Möller-Leimkühler, 2002), and stigma may have a large impact on help-seeking for depression (Barney et al., 2006). Depression is the fourth largest contributor to the global burden of disease (Hyman et al., 2006) and depressive disorders were the leading cause of years of healthy life lost due to disability in 2001 (Lopez et al., 2006). Unipolar depressive disorders are the third highest burden of disease in high-income countries (Lopez et al., 2006) and predicted to be one of the leading costs to the UK's National Health Service (NHS) in the future. The economic burden of depression also includes indirect costs, such as lost earnings. It is estimated that lost earnings alone cost both the UK and United States of America (US) economies tens of billions of pounds per year (MHF, 2010). Depression is also a disabling and distressing condition to live with and 16% of individuals with depression attempt suicide at some point in their lifetime (Chen and Dilsaver, 1996).

Consequences of Depression

There are a range of neuropsychiatric comorbidities that commonly occur in depression such as migraine, anxiety and insomnia (Mineka et al., 1998). Additionally, those with depression often develop or are diagnosed with other

physical conditions, such cardiovascular disease or type 2 diabetes mellitus, at an earlier age (Brown et al., 2005; Laake et al., 2014). Some of the increased risk for these conditions may be in part explained by behavioural characteristics associated with depression, for example sedentary lifestyle (Teychenne et al., 2010), reduced quality of life (Schram et al., 2009) and less social support (Cohen and Wills, 1985; Holahan and Holahan, 1987). In type 2 diabetes mellitus both successfully treating depression, and improving diabetes self-care behaviours, have not been consistently associated with improved prognosis (Katon et al., 2004; Ismail et al., 2007; Petrak and Herpertz, 2009; Van der Feltz-Cornelis et al., 2010). Therefore, it is unlikely that behavioural differences in depression are the only cause of the increased incidence of type 2 diabetes mellitus and cardiovascular disease. Some of the increased incidence of these conditions may instead be due to a shared disease aetiology (such as deregulation of the hypothalamic-pituitary-adrenal (HPA) axis or a raised inflammatory state); these are discussed in detail later.

Treatments

There are two main types of treatment for depression; these differ in their approach. The first, known as psychotherapy, includes: cognitive behavioural therapy (CBT), interpersonal therapy (IPT), counselling, and self-help materials. In the UK the National Institute for Health and Clinical Excellence (NICE) guidelines for the treatment of depression recommend that self-help therapies based on CBT should be the first line treatment for individuals diagnosed with mild or moderate depression (NICE, 2009). The aim of CBT is to enable those with depression and other disorders to identify unhelpful cognitions and behaviours so they can begin changing the cognitions and, in turn, change the associated behaviours (Ismail et al., 2010; Nevid, 2012). IPT is a highly structured form of psychotherapy where the problems individuals may face are explored under four headings of, bereavement and loss, role disputes, role transitions, and interpersonal deficits (Weissman et al., 2000; Gelder et al.,

2006). Both CBT and IPT have demonstrated efficacy in the treatment of depression (Beck, 1976; Weissman et al., 2000; Mufson et al., 2004).

The second type of treatment is antidepressants. Selective serotonin reuptake inhibitors (SSRIs) are the first line antidepressants, but other prescribed classifications of antidepressants include: tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), and serotonin-norepinephrine reuptake inhibitors (SNRIs) (BMA, 2010). These classes of antidepressants work by a variety of methods, the primary mode of action of TCAs is identical to SNRIs, which block the serotonin transporter (SERT) and the norepinephrine transporter (NET). This causes an elevation of the synaptic concentrations of these neurotransmitters, and thus enhances neurotransmission. MAOIs inhibit the activity of monoamine oxidase (MAO) – an enzyme that breaks down monoamine neurotransmitters such as serotonin, norepinephrine and dopamine. By inhibiting MAO, MAOIs increase the availability of monoamine neurotransmitters. The efficacy of each classification of antidepressant is broadly equivalent, however the side effects, toxicity and cost of each vary (Gelder et al., 2006). In the UK the NICE guidelines recommend a combination treatment of antidepressants with psychotherapy, such as CBT and SSRIs, as the most effective treatment for those with moderate depression (NICE, 2009). Antidepressants are also prescribed on their own to those suffering from severe depression and to those with moderate depression that is treatment resistant to CBT (NICE, 2009).

The third (less common) treatment for depression, electroconvulsive therapy (ECT) accounts for a small percentage of treatment for depression in the UK. This is used as a last intervention primarily for depression and only in order to achieve the rapid and short-term improvement of severe symptoms (NICE, 2009). ECT was popular in the 1940s and, though it is effective, its mode of action remains poorly understood (Gelder et al., 2006). As its use requires anaesthesia ECT should be only used when the potential benefits to the patient

justify the risks from use of anaesthetic as well as the risk of potential adverse effects of the ECT (NICE, 2009).

Controversies

The distinction between sub-threshold depression and clinically diagnosed depression and the validity of diagnostic tools is debated; sometimes individuals are classed as having sub-threshold depression as they fail to meet cut-off thresholds even though they are impaired (Kendell and Jablensky, 2003). The differences in the disorder boundaries for depression are more subjective and arbitrary (and perhaps less justified) than boundaries used for the diagnosis of other conditions such as anaemia or type 2 diabetes mellitus, which can be identified by measurable biomarkers (red blood cell count or glycated haemoglobin (HbA_{1c})). There have been many controversies with the publication of the DSM-IV, and more recently the DSM-V, however it is agreed that categories for depression are largely necessary for treatment and research (Brown and Barlow, 2005; Krueger et al., 2005).

Recently the term 'depression' or 'major depressive disorder' has come under considerable scrutiny. Classing all unipolar depressive disorders as one condition may be unhelpful as it is likely there are subtypes of depression that may be altogether different in their aetiology and pathogenesis (Hasler, 2010; Lichtenberg and Belmaker, 2010). There are several lines of evidence to support this, firstly, the efficacies of different treatments for depression are hugely varied depending on the individual and, secondly, some patients are resistant to antidepressants. Furthermore, mild depression is not improved by antidepressants (Fournier et al., 2010). Finally, there is conflicting evidence for and against several different theories for the neurobiology/pathogenesis of depression, outlined below. Heterogeneity of the aetiology and pathogenesis of depression may explain the variation in the effectiveness of different classes of antidepressants and psychotherapy.

Some antidepressants are associated with an increase in adiposity (Serretti and Mandelli, 2010). Increased adiposity causes increased secretion of inflammatory agents into the blood and increased systemic inflammation (Shelton and Miller, 2010). As the increased adiposity observed in depression, which may also be caused by the higher prevalence of sedentary behaviour, may partially explain the increased risk of type 2 diabetes observed in those with depression it is essential to adjust for adiposity when examining this relationship. It is not clear whether some antidepressants have direct pro- or anti-inflammatory effects or whether these associations are the result of changes in adiposity, as there is insufficient systematic evidence from randomised controlled trials or observation studies adequately adjusted for adiposity to support this (Hannestad et al., 2011).

Neurobiological Theories of Depression

The biological causes of the symptoms of depression are not completely understood. Depression in otherwise healthy patients is proposed to occur due to changes in the balance of neurotransmitters such as serotonin (5-HT), as well as alterations in endocrine and immune functions. There are several theories that seek to explain the neurobiological mechanisms of depression and, while each theory has strengths and weaknesses, it is likely that the mechanisms of depression are heterogeneous (Lichtenberg and Belmaker, 2010).

Depression is a familial disorder with a moderate heritability. Family, twin and adoption studies suggest that the heritability of depression is mostly due to genetic factors (Sullivan et al., 2000) and not, as was previously assumed, due to shared life stresses between parents and offspring or parental social behaviour.

Genetic factors account for 31-42% of the total susceptibility to depression with non-genetic (environmental) factors accounting for the remaining 58-69% (Sullivan et al., 2000). Though genetic factors are responsible for such a large proportion of the susceptibility to depression, understanding which genetic variants, alleles and loci are responsible has proved difficult largely due to the genetic heterogeneity of depression (Wray et al., 2010). This genetic heterogeneity of depression supports the theory that depression encompasses a variety of biological processes, the most significant of which are outlined below.

Inflammatory Hypothesis

An emerging hypothesis for the pathogenesis of depression is the inflammatory hypothesis, which was proposed in 1991 by Roger Smith (Smith, 1991) and is supported by several observations. Sickness behaviour occurs when the inflammatory response is activated in response to infection or chronic disease

(Dantzer and Kelley, 2007) and many of the symptoms of sickness behaviour are similar to those of depression; in particular, low-mood, anhedonia, fatigue, cognitive and psychomotor impairment (Dantzer et al., 2008). Depression is associated with inflammation (Raison et al., 2006; Frasure-Smith et al., 2007; Maes, 2008; Miller et al., 2009; Dowlati et al., 2010) even when no other medical disease is present (Miller et al., 2009). A meta-analysis of both cross-sectional community and clinical samples also reported positive associations of four inflammatory markers (CRP, IL-6, IL-1 and IL-1RA) and depressive symptoms in those that had a clinical diagnosis of depression (Howren et al., 2009). These cross-sectional associations cannot prove causality as some of the increased inflammation observed in depression may be due to common mediators, such as altered activity of the nervous system and sedentary behaviour, which occur in depression and can contribute to the release of IL-6 and C-reactive protein (CRP) (Henson et al., 2013).

In patients receiving interferon therapy for hepatitis C and patients receiving cytokine therapy for cancer, there is evidence that inflammation can induce depression (Capuron et al., 2002; Capuron and Miller, 2004). Furthermore, prospective studies have identified associations between increased inflammation and future risk of depressive symptoms; these suggest that inflammation may be involved in the aetiology of depression (Dantzer et al., 2008).

If increased inflammation is involved in the aetiology and pathogenesis of depression there are two main potential sources; the first is external psychological stimuli. For instance, in animal models external stress is associated with increased production of IL-1β, IL-6 and TNF-α (Maes, 2001). In humans there is some evidence from case-control studies that psychosocial stress can lead to acute increases in intracellular nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κβ), a key protein complex involved in the intracellular regulation of inflammation (Bierhaus et al., 2004). High concentrations of pro-inflammatory cytokines have effects on symptoms commonly associated with the construct of depression (Capuron et al., 2002).

Inflammatory cytokines can also influence other areas implicated in the pathogenesis of depression including neuroendocrine function, neurotransmitter metabolism and regional brain activity. For example, cytokines, including interleukin-6 (IL-6), interleukin-1 (IL-1) and tumour necrosis factor-α (TNF-α), alter the activity of the serotonin transporter (5-HTT), altering 5-HT uptake into pre-synaptic neurons (Dantzer et al., 2008).

The second possibility is that inflammation due to physical exposures peripheral to the central nervous system (CNS) is involved in the pathogenesis of depression. As systemic inflammation has been implicated in the pathogenesis of many chronic diseases, some have postulated this may also be the case in depression (Raison et al., 2006; Miller et al., 2009). Pro-inflammatory cytokines administered intravenously in the treatment of patients with hepatitis C often include depressive symptoms as side effects (BMA, 2010). In particular interferon-alpha (IFN-α) induces changes in inflammatory biomarkers; these changes are greater in those patients that develop depression compared to those that do not (Capuron and Miller, 2004). IFN-α administration is positively associated with increased concentrations of IL-1, interleukin-1 receptor antagonist (IL-1RA), IL-6, interleukin-8, monocyte chemotactic protein-1 (MCP-1) and TNF-α (Capuron and Miller, 2004).

Systemic inflammation may have an effect on the brain through neuroinflammatory mechanisms. Pro-inflammatory cytokines produced outside CNS are not able to diffuse directly across the blood brain barrier, but they can diffuse into the brain through blood brain barrier deficient areas (Banks et al., 1995). IL-1β, TNF-α and IL-6 can also be transported into the brain through endothelial cell transporters and can influence the brain by activating signalling in the afferent vagus nerve, which alerts the CNS to the presence of inflammation (Goehler et al., 2000; Turrin and Rivest, 2004; Banks, 2005; Pavlov and Tracey, 2005). The indoleamine-2,3 dioxygenase pathway is activated by inflammation

and breaks down tryptophan – an amino-acid which is the precursor of 5-HT. In patients with depression, (both TNF-α and non-TNF-α induced) serum concentrations of 5-HT are decreased and serum tryptophan metabolites are increased (Bonaccorso et al., 2002; Bonaccorso et al., 2002; Wichers et al., 2005; Myint et al., 2007). Similar results have been reported from cerebrospinal fluid (Raison et al., 2010). The indoleamine-2,3 dioxygenase pathway has also been implicated in causing brain inflammation through the production of neurotoxic tryptophan metabolites (Capuron and Miller, 2004; Maes, 2008) which may cause neurological damage and symptoms of depression (Myint et al., 2007). Additionally, pro-inflammatory cytokines such as IL-6 have been associated with depression and can cross the blood brain barrier. Finally, pro-inflammatory chemokines such as MCP-1, also associated with depression, may have a role in altering the permeability of the blood brain barrier (de Vries et al., 1996). These mechanisms explain how increased concentrations of peripheral cytokines are able to induce physiological responses in the CNS and behavioural changes (Qin et al., 2007).

A large prospective study (n = 1037) of the effects of childhood adversity, a major risk factor for adult depression, demonstrated an increased risk for higher concentrations of CRP and depression (Danese et al., 2009). A smaller recent study in a non-depressed population (n = 38) also reported that childhood adversity is prospectively associated with higher concentrations of C-reactive protein (CRP) in early adult life (Hartwell et al., 2013). However, this was a secondary analysis of a larger study and therefore may have been underpowered. By reducing glucocorticoid receptor (GR) function, pro-inflammatory cytokines also affect the activity of the HPA axis and neurogenesis in the hippocampus. The HPA axis and neurogenesis have been reported to be involved in the pathogenesis of depression and the HPA axis is also affected by childhood adversity, both are discussed in detail later.

One of the effects of the acute phase response is to alter glucose homeostasis by increasing glucose production and reducing glucose clearing (insulin resistance), thus increasing blood sugar (Weissman, 1990). Therefore, a persistent acute phase response may explain some of the increased concentrations of blood glucose in patients with depression and type 2 diabetes mellitus. The glucose concentration of the brain is typically 15%-20% of that in the blood and the acute effects of hyperglycaemia on the brain include mild cognitive impairment (Cox et al., 2005). Additionally, the prevalence of depression in those with type 2 diabetes mellitus is 2-3 times greater than the prevalence in the general population (Anderson et al., 2001). In these individuals chronic hyperglycaemia is associated with microvascular disease, and it has been proposed that high blood glucose concentrations may have similar effects on the microvasculature in the CNS (Qiu et al., 2010). This damage may occur long before the development of type 2 diabetes and may contribute to depression as well as cognitive impairment.

The efficacies of classes of antidepressants may vary according to inflammatory profile, and some antidepressants may also have anti-inflammatory actions (Kenis and Maes, 2002; Hannestad et al., 2011, Uher et al. 2014). Individuals with depression and high concentrations of CRP have been shown to respond better to antidepressants than individuals with lower CRP concentrations (Harley et al., 2010). In contrast, those with lower concentrations of IL-6 or TNF-α respond better to antidepressants than those with higher circulating concentrations (Lanquillon et al., 2000; Eller et al., 2008). There is limited evidence, from controlled studies with small sample sizes, that antidepressant treatments may also be associated with a reduction in inflammation. In one study those that responded best to the antidepressant treatment showed a significantly greater decrease in TNF-α than individuals that did not (Lanquillon et al., 2000). Additionally, antidepressants have been shown to reduce systemic concentrations of CRP in a mouse model, irrespective of any improvement in depressive symptoms (O'Brien et al., 2006).

Current evidence suggests the inflammatory hypothesis for depression does not account for all depression. Further adding weight to the notion that, while inflammatory processes may be responsible for some subtypes of depression, it is possible that they are less or totally uninvolved in others. While many studies have adjusted for potential confounders, there is a risk that positive associations observed between inflammation and non-interferon-induced depression are coincidental and can be explained by residual confounding, discussed later.

Hypothalamic-Pituitary-Adrenal Axis Hypothesis

A hypothesis that links well with the inflammatory hypothesis of depression is the HPA axis model of depression. The HPA axis is a complex set of interactions among the hypothalamus, the pituitary and adrenal glands that control reactions to stress and regulate, among other things, inflammation, mood and metabolism.

The HPA axis is activated by various acute phase cytokines, such as IL-1, TNF-α and IL-6, in response to infection and is involved in sickness behaviours (Bethin et al., 2000). The HPA axis is closely linked with the regulation of inflammation and glucose homeostasis in response to stress stimuli. It releases and up-regulates stress hormones to effect inflammatory responses, and alter glucose metabolism, as part of the normal acute phase response to infection.

Under normal HPA axis function, serum and salivary cortisol concentrations follow a distinct daily pattern – the circadian cortisol cycle. Cortisol rises after awakening in the morning and reaches a peak 30-45 minutes afterwards. After this time cortisol concentrations gradually decline throughout the awake period, reaching a low during the middle of the night (Deuschle et al., 1997).

Stress, physical activity, illness and the circadian rhythm all influence the release of corticotrophin releasing hormone (CRH) from the hypothalamus. CRH stimulates the secretion of adrenocorticotropic hormone (ACTH) from the anterior lobe of the pituitary gland. The adrenal cortex, part of the adrenal gland, produces the glucocorticoid hormone cortisol in response to ACTH. Glucocorticoids suppress CRH and ACTH production in the hypothalamus and pituitary in a negative feedback cycle (Deuschle et al., 1997).

Between 20-80% of depressed individuals have some form of HPA hyperactivity (Stetler and Miller, 2011). One of the cheapest and least invasive methods of measuring HPA axis activity is measurement of salivary cortisol concentration. In individuals with depression, waking salivary cortisol concentration is increased compared to controls (Pariante and Lightman, 2008). Some neuroimaging studies have reported adrenal and pituitary gland volumes, measures of HPA axis function, are also greater in depression (Kessing et al., 2011). There is also evidence that CRH may play a role in the pathogenesis of depression. Elevated concentrations of CRH have been reported in depressed patients, while post-mortem studies have reported increased numbers of CRH secreting neurons in the brain (Nemeroff et al., 1984; Nemeroff et al., 1988; Nemeroff, 1996). CRH also produces a number of physiological and behavioural alterations that resemble the symptoms of depression, including decreased appetite, disrupted sleep, decreased libido, and psychomotor alterations (C. B. Nemeroff, 1996).

Early life-stress, a risk factor for increased depression and inflammation later in life, has a significant impact on the programming of the HPA axis. In animal models acute exposure to mild or moderate stress in early life enhances HPA axis regulation (Macri et al., 2009; Lyons et al., 2010) while exposure to extreme prolonged stress induces a hyper-reactive HPA axis that may underlie increased vulnerability to future stress (Sanchez et al., 2001). There is some evidence from observational studies that these polarised effects of early life stress may extend to humans. In the adoption model of early life stress a small observational study

of 11-12 year olds identified altered activity of the stress hormone cortisol in those with moderate early life stress (Gunnar et al., 2009). Additionally, a prospective controlled study reported altered secretion of CRH, ACTH and cortisol are common in those with a history of childhood trauma or adversity (Heim et al., 2008).

As many hormones and peptide-releasing agents also function as neurotransmitters in the brain, the roles of other hormones in the pathophysiology of depression, and as potential therapeutic targets, are also being investigated. Clinical studies of the hormone vasopressin, which is involved the HPA axis and stress-mediated behaviours, suggest abnormalities in vasopressin activity may be involved in the alterations to HPA axis function observed in depression (Griebel and Holsboer, 2012). Other hormones that function as neurotransmitters such as 5-HT and norepinephrine are discussed in more detail later under the monoamine deficiency hypothesis.

As with the inflammation theory for depression, evidence for activation of HPA axis in depression is inconsistent. This is demonstrated by the CRH stimulation test which, though a sensitive measure of HPA axis dysfunction, has a relatively low specificity for identification of depression (Heuser et al., 1994). This implies that HPA axis dysfunction does not always cause depression, that other factors must also be involved in its aetiology and, furthermore, supports the theory for the heterogeneity of the aetiology and pathogenesis of depression.

There are several methods used to measure HPA axis activity, including: waking salivary cortisol, 24-hour urine free cortisol (UFC) levels, the dexamethasone suppression test (where morning cortisol concentrations are measured following administration of dexamethasone the evening before sampling), measurement of adrenal gland size or volume, the dexamethasone-CRH test (where low dose dexamethasone is administered for 2 days and then morning cortisol is

measured following CRH infusion). Failure to suppress cortisol with the dexamethasone suppression test or the dexamethasone-CRH test demonstrates an inability of the HPA axis to terminate the stress response appropriately, which may be due to HPA axis dysfunction.

It would be interesting to simultaneously investigate both the relationship of HPA axis function and inflammation and depression in type 2 diabetes mellitus in one study. However, due to the circadian nature of HPA axis function, measurement requires either administration of a drug, controlling for waking time and/or testing at specific times of the day. This is difficult in large epidemiological studies and the alternative of magnetic resonance imaging (MRI) or computerised tomography (CT) scans to determine adrenal gland size are not cost effective.

Other Biological Theories

Perhaps the original neurobiological theory of depression is the monoamine deficiency hypothesis. This postulates that a decrease in basal levels of 5-HT, norepinephrine and dopamine underlie the pathogenesis and maintenance of depressive symptoms.

Norepinephrine and 5-HT are both neurotransmitters and are widely distributed in the brain. As a result they are involved in a range of neuropsychological processes, including depression. A number of abnormalities in 5-HT, its precursors and metabolites are common in depression; most convincing are systemic decreases in tryptophan concentrations, an amino-acid precursor of 5-HT (Cowen et al., 1989). Reductions in the concentrations 5-hydoxindoleacetic acid, a 5-HT metabolite, in CSF have also been reported (Brown and Linnoila, 1990). Less is known about dopamine and norepinephrine in depression in part because they are harder to measure. However, in those that have attempted

suicide CSF concentrations of homovanillic acid (a metabolite of dopamine) are also reduced, while in suicide victims homovanillic acid concentrations are elevated in the frontal cortex (Dunlop and Nemeroff, 2007). Dopamine receptor binding has been studied in depression, however reports of binding abnormality in post-mortem studies of depression are inconsistent, and the results may be confounded by previous antidepressant therapies. Reductions in dopamine, its precursors and metabolites, are consistent with two symptoms of depression, anhedonia and psychomotor retardation (Dunlop and Nemeroff, 2007).

From a meta-analysis of healthy adult volunteers, artificially reducing 5-HT concentrations (by means of a restricted tryptophan diet) does not induce depressive symptoms, although changes in memory and learning have been reported (Ruhe et al., 2007). Conversely in previously depressed patients, who are no longer receiving medication, depressive symptoms return in about 30% when receiving the restricted tryptophan diet. These depressive symptoms remit once normal diet is resumed (Delgado et al., 1990; Smith et al., 1997; Ruhe et al., 2007). Similarly reducing dopamine concentrations does not have depressive effects in healthy volunteers but does in previously depressed patients who are no longer receiving medication (Berman et al., 1999). This suggests that, while reduced 5-HT and dopamine concentrations may play a role in some cases of depression, depletion of 5-HT and dopamine alone do not cause depression in healthy individuals.

In the neuron, excess neurotransmitter molecules (such as norepinephrine, dopamine, and 5-HT) are deactivated by MAO, which acts like a switch to prevent their accumulation. If the protein expression of MAO is increased, concentrations of 5-HT, norepinephrine and dopamine are reduced (Figure 1). The monoamine deficiency hypothesis is supported by the effectiveness of MAOIs for the treatment of depression, discussed earlier (Mann, 2005; BMA, 2010).

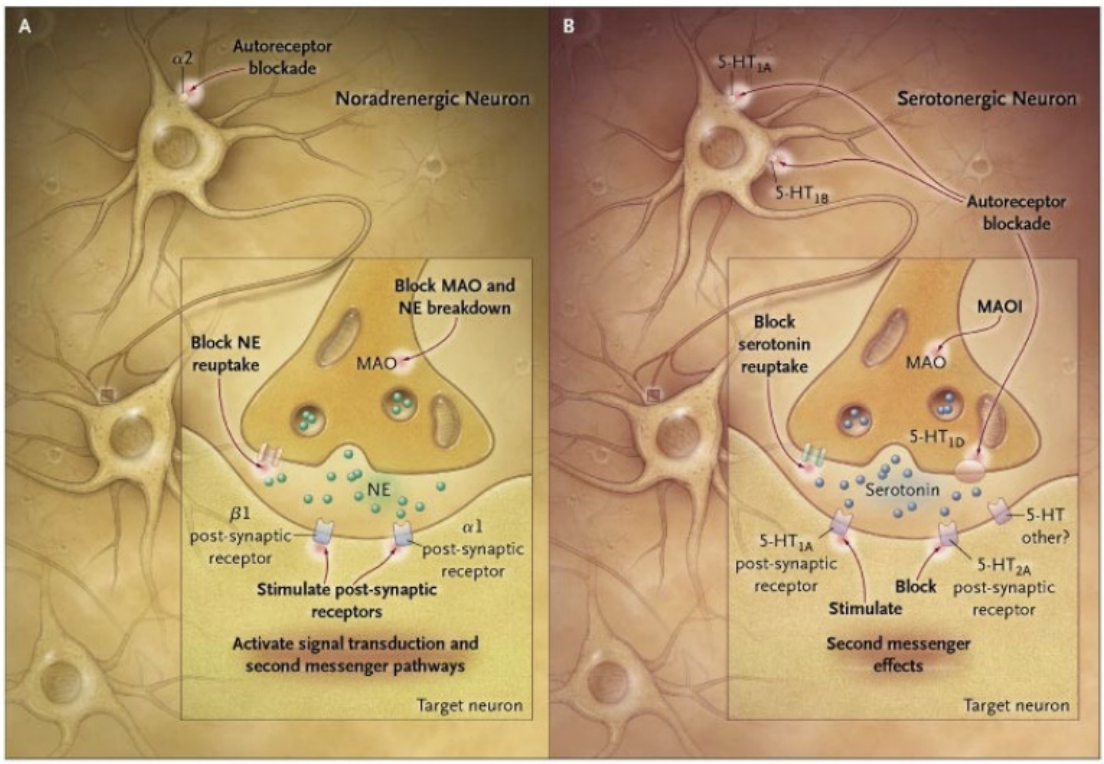


Figure 1.1: Effects of antidepressants on A – The Noradrenergic System, B – The Serotonergic System, (Reproduced with permission from (Mann, 2005), Copyright Massachusetts Medical Society).

Recent advances in imaging technology have allowed measurement of noradrenergic and serotonergic activity in the living human brain, offering the potential for increased precision and detail (Cowen, 2008). Positive emission tomography (PET) studies indicate that MAO is increased in prefrontal cortex, anterior cingulate cortex, posterior cingulate cortex, caudate, putamen, thalamus, anterior temporal cortex, midbrain, hippocampus, and parahippocampus of depressed patients (Meyer et al., 2006). PET studies of 5-HTT and 5-HT receptors in depression are, like studies of dopamine receptor binding, inconsistent. In some studies of depressed patients lower 5-HTT binding is observed in the midbrain (Selvaraj et al., 2007).

Like the inflammatory hypothesis of depression, the main limitation of this theory is that medications that target these systems (the MAO neurotransmitter systems) do not have universal efficacy (Mann, 2005). Futerhmore, although

brain imaging research is in its infancy, there are a great deal of inter-study inconsistencies in results reported (Cowen, 2008). This suggests that abnormalities in 5-HT and norepinephrine signalling are not central to the pathogenesis of all cases of depression, further supporting the idea of the heterogeneity of the pathogenesis of depression.

Another theory is the neurotrophic model. This postulates that the loss of brain-derived neurotrophic factor (BDNF) plays a major role in the pathophysiology of depression. The principle evidence supporting this model is that psychosocial stress leads to a reduction in BDNF (Dranovsky and Hen, 2006), impairing neurogenesis (cell proliferation) in the hippocampus (an area of the brain that is involved in controlling emotionality) and increasing hippocampal atrophy (Groves, 2007). As neurogenesis is largely confined to two regions in the healthy adult brain, the subventricular zone of olfactory bulb and the dentate gyrus in the hippocampus, it is now believed that BDNF stimulated cell proliferation in these loci may be involved in maintaining balanced mood (Groves, 2007). This theory fits with the HPA axis and inflammatory hypotheses of depression as glucocorticoids and pro-inflammatory cytokines including IL-6, TNF-α and IL-1β also inhibit neurogenesis and may contribute to neurodegeneration (Sapolsky, 2004; Sorrells and Sapolsky, 2007).

The role of BDNF in depression is still not clear, while some antidepressants lead to decrease of BDNF in mouse models, increased concentrations of BDNF have been linked with behavioural despair in the mouse forced swim model (Eisch et al., 2003). Suppression of the BDNF receptor TrkB has been shown to have antidepressant like effects (Eisch et al., 2003) but the receptor is also associated with efficacy of antidepressant treatments (Li et al., 2008). Most recently research has demonstrated opposite functional properties of BDNF in the ventral tegmental area (VTA)-to-nucleus accumbens (Nac) reward pathway to the properties reported in hippocampus (Berton et al., 2006). This suggests that the

effects of increases or decreases of BDNF on depression are complex and differ according to which specific regions of the brain are affected.

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain and impaired GABAergic transmission has been implicated in depression. MRI studies have shown reductions in concentrations of GABA in the pre-frontal and occipital cortex in depression (Hasler et al., 2007). These may be due to a reduction of the density or size of GABAergic interneurons that have been reported (Rajkowska et al., 2006), or alternatively, due to the acute effects of stress, which appears to be associated with the down-regulation of pre-frontal GABAergic transmission (Hasler et al., 2010). Although they do not specifically target GABA, both SSRIs and ECT can increase concentration GABA in depressed patients (Sanacora et al., 2010). However benzodiazepines, which are positive allosteric modulators (they bind to benzodiazepine binding sites of the GABA receptor complex increasing their affinity for endogenous GABA), do not have consistent effects on depressive symptoms (Barbui et al., 2011) and some studies have shown no differences in GABA concentrations in depressed patients (Hasler et al., 2005).

Several lines of evidence also suggest dysfunction of the glutamatergic transmission in depression. Glutamate dysfunction was first implicated in the pathophysiology of depression by studies that identified loss of cortical glial cells, which play a key role in regulating glutamate activity in the synapse, in depression (Ongur et al., 1988). Subsequently studies of glutamate dysfunction have identified reduced glutamate concentrations in plasma serum and CSF (Sanacora et al., 2008). MRI studies have detected abnormal glutamate concentrations in those with depression (Hasler et al., 2007) and glutamate N-methyl-D-aspartate (NDMA) receptor antagonist produced rapid antidepressant effects in a group of patients with treatment-resistant depression (Zarate et al., 2006). Several inhibitors of glutamate release including riluzole and lamotrigine also have antidepressant properties (Sanacora et al., 2007).

Type 2 Diabetes Overview

Clinical Characteristics

Type 2 diabetes mellitus is a chronic condition that is characterised by persistent hyperglycaemia and caused by a decrease in insulin stimulated glucose uptake (Holt et al., 2011). The condition is diagnosed using a number of physical symptoms in conjunction with at least one positive biochemical assay for raised blood glucose (WHO, 2006; Holt et al., 2011). In the absence of any physical symptoms, diagnosis can only be made following a repeat of the biochemical assay with a positive result. There are two tests for blood glucose that are used as a diagnosis for type 2 diabetes mellitus; a random blood glucose > 11.1 mmol l^{-1}, or a fasting blood glucose > 7.0 mmol l^{-1} (WHO, 2006). A third diagnostic test, the glucose tolerance test (GTT), is used if the diagnosis is still unclear, for example, if the results of a random blood glucose test are close to the threshold and physical symptoms of diabetes are reported. Following an overnight fast, a blood sample is taken before administration of 75 mg glucose orally as a 250-330ml aqueous solution (Holt et al., 2011). If blood glucose is raised above 11.1 mmol l^{-1} following this test a diagnosis of diabetes can be made, if it is above 7.8 mmol l^{-1} this is an indicator of impaired glucose tolerance (IGT). Since July 2011 glycated haemoglobin (HbA_{1c}) has been used in the UK as a diagnostic tool for type 2 diabetes mellitus, with a venous HbA_{1c} of 48 mmol mol^{-1} (International Federation of Clinical Chemistry [IFCC] units) or 6.5% (Diabetes Control and Complications Trail [DCCT] units) or greater signifying type 2 diabetes mellitus. Importantly an HbA_{1c} of less than 48 mmol mol^{-1} does not rule out type 2 diabetes mellitus where it has been confirmed with glucose tolerance tests (WHO, 2011). HbA_{1c} is also used as a proxy measure of average plasma glucose concentration for the management of type 2 diabetes mellitus and is used to determine suitable treatments.

Epidemiology

There is a worldwide epidemic of type 2 diabetes mellitus. The condition currently affects over 2 million people within the United Kingdom and an estimated 311 million worldwide, accounting for 90% of all types of diabetes (WHO, 2011). In the UK between 25-50% of diabetes is undetected and so there are at least an additional five hundred-thousand undiagnosed individuals with type 2 diabetes mellitus (Forouhi et al., 2006).

Patients do not seek treatment for a number of reasons. There is a well-known gender bias where males appear less likely to seek treatment when experiencing symptoms, in particular those related to diabetes, such as fatigue (Macintyre et al., 1996; Kroenke and Spitzer, 1998). However, during the early stages of type 2 diabetes mellitus, patients are often asymptomatic so are not prompted to seek treatment. There are no plans in the UK to introduce national screening for diabetes, as the benefits of screening have not been proven by randomised control trials (RCTs). Those at high risk for type 2 diabetes mellitus are however screened within the NHS Health Check Programme and national vascular risk assessment programme for those aged 40-74 years (NICE et al., 2012). This means that though many of the sample population will have been diagnosed with type 2 diabetes early in the course of the condition, there may be a selection bias for those with high risk of cardiovascular disease (particularly those who are more obese). Female subjects may also have a shorter duration of undiagnosed diabetes than male subjects at diagnosis and recruitment; this is discussed in more detail in Chapter 7.

It is estimated that approximately 10% of the annual NHS budget was spent on diabetes in 2006 (Department of Health, 2006), an estimated £9 billion per annum in direct costs, making it one of the most expensive medical conditions in the UK. Inpatient treatment of diabetes complications accounts for the largest proportion of these direct costs. Diabetes is estimated to have cost the UK an additional £13 billion in indirect costs in 2010/2011 and the economic burden of the condition is likely to increase; type 2 diabetes mellitus predicted to cost the

UK a total of £35.6 billion a year by 2036 (Hex et al., 2012). The known risk factors for type 2 diabetes mellitus are ageing, obesity, a sedentary lifestyle, Asian and African ethnicity and 40 genetic allelic loci to date (Feero et al., 2010).

Comorbidity & Complications

There is no cure for type 2 diabetes mellitus and it has a large number of associated complications predominantly related to circulatory problems (Turner et al., 1998). Complications can be categorised according to the type of vasculature affected, microvascular and macrovascular. Macrovascular complications of type 2 diabetes mellitus include cerebrovascular accident (CVA), cardiovascular disease (CVD) and peripheral artery disease (PAD). CVD is the main cause of mortality in those with metabolic syndrome (Laakso, 1999; Holt and Kumar, 2009), with those suffering from type 2 diabetes mellitus having up to three times the risk compared with the general population (Wilson and Culleton, 1998).

The microvascular complications associated with type 2 diabetes mellitus are diabetic retinopathy, nephropathy and neuropathy (Holt et al., 2011). Depression and cognitive impairment have been proposed as microvascular complications due to the similarity between tissues of the CNS and ocular tissue and links between cerebral micro-bleeds and dementia (Qiu et al., 2010). Those with type 2 diabetes mellitus are also much as three times as likely as the general population to be depressed (Anderson et al., 2001). Though some studies have reported the incidence of depression is no higher in those with type 2 diabetes mellitus than those without (Brown et al., 2006), there is substantial evidence to suggest that, in some cases at least, the incidence of depression is higher in diabetic populations (Knol, 2006; Engum, 2007; Maraldi, 2007; Golden, 2008); the mechanisms underlying this association are poorly understood.

Treatments

There are several treatments that are used to manage type 2 diabetes mellitus by reducing average blood sugar, which reduce the incidence of some complications and mortality (Holt and Kumar, 2009). The first line treatment for new-onset type 2 diabetes mellitus is nutritional advice from a healthcare professional (with expertise in nutrition) to encourage a diet consisting of low-glycaemic-index sources of carbohydrates, low fat dairy products and limited intake of foods containing saturated and trans fats (NICE, 2009). A recent proof of concept study even suggests that for some individuals a very low-calorie diet for rapid weight loss can even reverse type 2 diabetes mellitus (Lim et al., 2011). Second line treatments are metformin, sulfonylureas, acarbose and third line treatments include dipeptidyl peptidase-4 (DPP-4) inhibitors, thiazolidinediones, glucagon-like peptide-1 (GLP-1) inhibitors and insulin. These can be prescribed to the patient sequentially as necessary (NICE, 2009). Blood pressure therapy including angiotensin-converting-enzyme (ACE) inhibitors, calcium channel blockers, alpha-blockers, beta-blockers, diuretics and lipid lowering medications such as statins and ezetimibe are also prescribed at lower thresholds (NICE, 2009). For those morbidly obese type 2 diabetes mellitus patients that have had bariatric surgery, many have significant improvement in blood glucose and some have improved enough to have normal glucose homeostasis (Buchwald et al., 2009).

Controversies

The treatment for type 2 diabetes mellitus consists of dietary advice and medications designed to lower blood glucose. Lowering blood glucose appears to be particularly effective at reducing microvascular complications (Turner et al., 1998), but less so at reducing macrovascular disease. Macrovascular disease may be treated more effectively with low dose anti-inflammatory medications, such as aspirin (Kirkman et al., 2006), which is prescribed prophylactically to those with type 2 diabetes mellitus and risk factors for cardiovascular disease (NICE, 2009). The benefits of these medications in reducing macrovascular disease may

be due to effects of adiposity and diet on vascular inflammation and oxidative stress (Lyon et al., 2003). Evidence suggests that, while the glucose lowering medications do improve the rate of some complications in those with type 2 diabetes mellitus, those with good glycaemic control through diet and medication are still at a great risk of diabetes complications and earlier mortality (Turner et al., 1998; UKPDS, 1998). It may be that other processes occurring in type 2 diabetes mellitus, such as inflammation, which may be the cause of macrovascular complications, may also be a causal factor of the high blood sugar levels rather than a consequence. For example, it is known that higher concentrations of advanced glycation endproducts (AGEs) occur in those with worse glucose control (Brownlee, 2001). These AGEs are known to have an inflammatory effect (Bierhaus et al., 2003; Bierhaus et al., 2004; Chavakis et al., 2004) and so by reducing the concentration of glucose in the blood, and therefore the concentrations of these AGEs, some improvement in diabetes complications would be expected. However, there are many other inflammatory mechanisms that may also be involved in the pathogenesis of type 2 diabetes mellitus and common complications (Pickup, 2004), if these inflammatory mechanisms continue even while blood sugar levels are controlled this would go some way to explain the complications that still occur in those with well managed type 2 diabetes mellitus or pre-diabetes IGT (Singleton et al., 2003).

As has been proposed in depression, there may well be different subtypes of type 2 diabetes mellitus which differ in their aetiology. For example, while increased adiposity is a known risk factor for type 2 diabetes mellitus (Wang et al., 2005) and extreme weight loss can return glucose homeostasis to normal levels (Lim et al., 2011), many of those with type 2 diabetes mellitus are not overweight.

Biological Mechanisms of Type 2 Diabetes Mellitus

Metabolic Mechanisms

Diabetes is a progressive metabolic condition, caused by dysfunction of glucose metabolism, resulting in chronic hyperglycaemia. Specifically, dysfunction involves defects in insulin secretion by pancreatic beta cells coinciding with peripheral insulin resistance in liver and muscle and adipose tissues (Bajaj and DeFronzo, 2003; Donath and Shoelson, 2011). Insulin resistance does not in itself cause type 2 diabetes mellitus, as in the normal popualtion approximately 20% of individuals are insulin resistant but do not suffer from raised blood glucose. It is argued that this is due to compensation by the pancreatic islets, which increase insulin production to counteract initial insulin resistance (Bajaj and DeFronzo, 2003), and that it is only after pancreatic islet cells have reached the limit of insulin secretion, that insulin deficiency, (caused in part by an increase in plasma FFAs), IGT and type 2 diabetes mellitus develop.

Inflammatory Processes

A well-recognised inflammatory process underlies the metabolic condition of type 2 diabetes mellitus, part of the innate immune response (the initial non-adaptive immune reaction of the body to tissue stress or damage) the acute phase response was first suggested as a long-term process that was active during type 2 diabetes mellitus over 15 years ago (Pickup et al., 1997). During the process acute phase proteins increase in concentration in specific tissues and systemically through blood circulation; this acute phase response becomes chronic in long-term conditions such as arthritis and cancers (Coussens and Werb, 2002).

Numerous prospective cohort and cross-sectional studies have identified raised concentrations of acute phase proteins and cytokines, including IL-6 and CRP, in

patients with type 2 diabetes mellitus (Snijder et al., 2001; Freeman et al., 2002; Thorand et al., 2003; Pickup, 2004). Additionally, circulating markers of inflammation such as IL-6, the major cytokine mediator of the acute phase response, are strongly associated with the development of type 2 diabetes mellitus (Freeman et al., 2002; Pickup, 2004). Elevated concentrations of the acute phase protein CRP are also a risk factor for cardiovascular complications in patients with type 2 diabetes mellitus (Ridker et al., 2002) and have been suggested as a cause of it (Li and Fang, 2004). These molecules which, in elevated systemic concentrations, are currently known biomarkers for future risk of both diabetes and diabetic complications are, as of yet, untested as biomarkers of the potential psychological complications of type 2 diabetes mellitus such as depression and cognitive impairment.

Some anti-inflammatory agents may improve both glycaemic control and systemic inflammation in type 2 diabetes mellitus, but their effects have not yet been fully explored (Hovens et al., 2008). The methods of anti-inflammatory agent administration that have been tested in type 2 diabetes mellitus are oral (aspirin and salsalate), subcutaneous injection or intravenous drip (anakinra and tocilizumab) (Larsen et al., 2009; Ogata et al., 2011). Aspirin works by inhibiting cyclooxygenase-1 (COX-1) (an enzyme involved in the synthesis of prostaglandins that mediate inflammatory reactions stimulated by interleukins such as IL-1). Salsalate is believed to work in a similar way by inhibiting the production and release of prostaglandins, and thus the inflammatory response. There is also some evidence to suggest that salicylates (including aspirin and salsalate) may have an additional mode of action – through the inhibition of the transcription factor NF-κβ (Stevenson et al., 1999; Wu, 2000). Anakinra is an IL-1 receptor antagonist and competitively binds to the IL-1 receptor, blocking the activity of IL-1 (Arend, 2002), this in turn prevents the cyclooxygenase cycle. Tocilizumab works by competitively binding IL-6 receptors and inhibiting the pro-inflammatory action of IL-6.

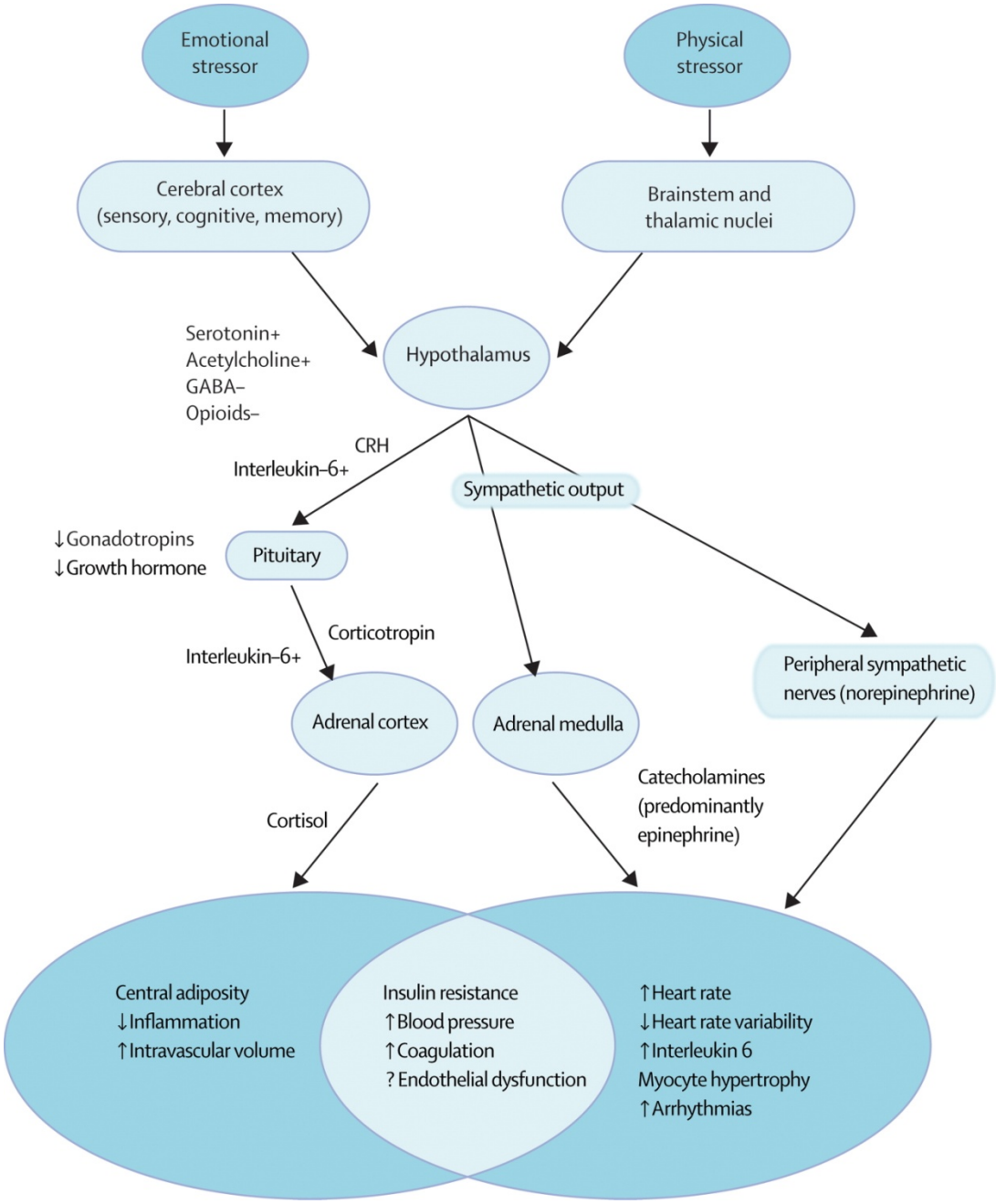


Figure 1.2: Metabolic and Cardiovascular effects of the stress response, (Reprinted from The Lancet, 370, Brotman DJ, Golden SH, Wittstein IS, The cardiovascular toll of stress, Pages 1089-1100, Copyright (2007), with permission from Elsevier).

Some of these anti-inflammatory agents have demonstrated efficacy in glycaemic control and can dramatically improve HbA_{1c} (Ogata et al., 2011). The benefits of lowering both HbA_{1c} and also inflammation could have greater health benefits than purely lowering HbA_{1c}, particularly in individuals who have had poorly controlled diabetes for several years (Turner et al., 1998; UKPDS, 1998). For such individuals it is known that lowering HbA_{1c} is less successful at reducing diabetic complications, and research has led to the theory that this may be due to a reinforcing inflammatory condition (Pickup, 2004). As well as reducing diabetic complications, lowering inflammation may also help to improve other symptoms associated with diabetes, such as fatigue (Maes et al., 2012). There is limited evidence to suggest these drugs are effective at improving glycaemic control.

Comorbid Depression and Type 2 Diabetes Mellitus

A Bi-directional Relationship

Most epidemiological research on the prevalence of depression in type 2 diabetes mellitus has used self-report questionnaires, such as the PHQ-9, to identify depressive symptoms or possible cases of depression. It is known that there is a bi-directional association between type 2 diabetes mellitus and depression. Rates of depression are at least two, but as much as three, times greater when compared to those in the general population or those with no chronic disease (Anderson et al., 2001; Knol et al., 2006; Engum, 2007; Maraldi et al., 2007; Golden et al., 2008). Depressive episodes are typically longer in those with type 2 diabetes mellitus (Fisher et al., 2008). The causes for this more persistent recurring depression are unclear but may be due to the burden of living with and managing type 2 diabetes mellitus, a chronic condition that requires daily management (Winkley, 2010), and/or a shared inflammatory aetiology (Laake et al., 2014).

Psychological Model of Depression

This model explains the association between depression and type 2 diabetes mellitus through behavioural differences between those with depression compared to those without depression. Poor self-care behaviours, which are a facet of depression, poor diet (Ciechanowski et al., 2000), sedentary lifestyle (Teychenne et al., 2010), reduced quality of life (Schram et al., 2009), and less social support (Cohen and Wills, 1985; Holahan and Holahan, 1987) may explain the 37-60% increased risk of incident type 2 diabetes mellitus in those with depression (Knol et al., 2006; Mezuk et al., 2008). Furthermore, these behavioural differences may also lead to differences in the self-management of established diabetes, such that the differences in glycaemic control may account for the increased mortality and earlier onset of diabetes complications in those with depression and type 2 diabetes mellitus (Hermanns et al., 2005). As

depression is associated with late diabetes complications, the 15-24% increased risk of incident depression observed in type 2 diabetes (Mezuk et al., 2008; Nouwen et al., 2010) may be due to the burden of living with and managing type 2 diabetes and coping with complications (Winkley, 2010).

Biological Model of Depression

This model explains the association between type 2 diabetes mellitus and depression as a result of a shared physiological aetiology and pathogenesis that includes differences in HPA axis regulation and inflammation (Figure 1.3). As previously discussed, increased inflammation is a risk factor for the development of type 2 diabetes mellitus and has been proposed as being involved in the pathogenesis of type 2 diabetes mellitus and some forms of depression.

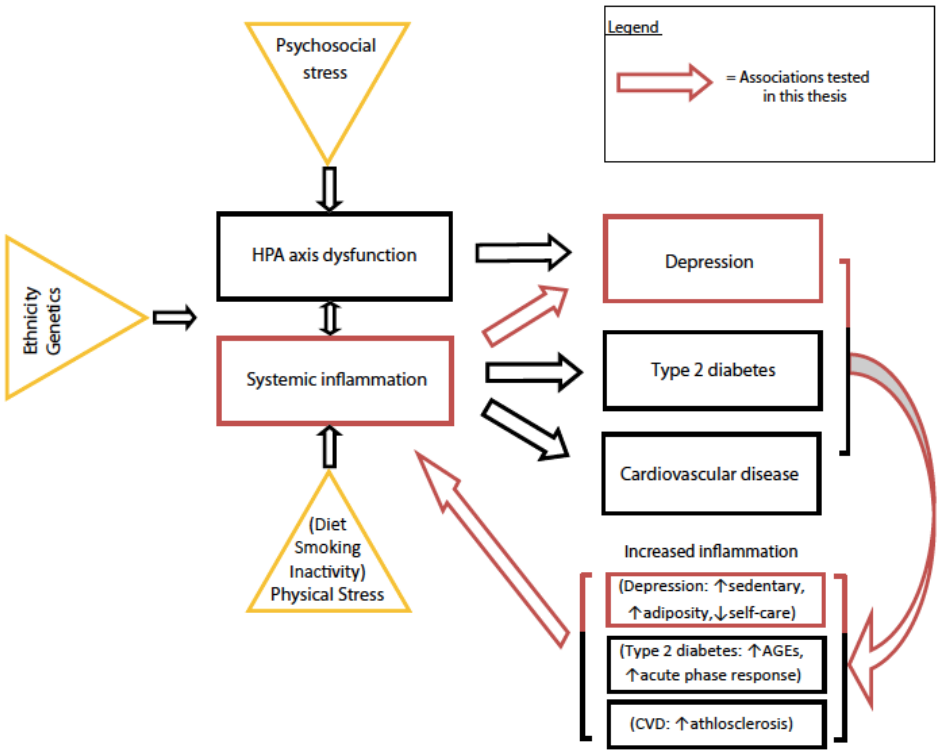


Figure 1.3: the HPA axis-inflammatory model of type 2 diabetes mellitus & depression, (adapted from Pickup, 2004).

A range of risk factors relevant to type 2 diabetes mellitus may be implicated in the increased risk of depressive symptoms. Manifestation of diabetes, occurrence of complications, poor glycaemic control (blood sugar), insulin therapy, hypoglycaemia, increased inflammation and HPA axis disruption have all been postulated as potential risk factors for depression in type 2 diabetes mellitus. To date the only diabetes specific risk factors for depression to have been identified are diabetes complications, poor glycaemic control and insulin therapy (Hermanns et al., 2005).

The associations between depression and type 2 diabetes mellitus are also confounded by age. The prevalence of type 2 diabetes increases with age and, though depression is not a normal part of ageing, the prevalence of depression is also higher in older people – in particular those with another comorbid illness (Moussavi et al., 2007). In type 2 diabetes mellitus depressive symptoms are also more common in younger individuals, supporting evidence that depression increases the risk of diabetes (Fisher et al., 2008).

Comorbidity & Complications

The management of diabetes, when compared to other chronic health problems, requires additional education and complex self-care. This may explain why depression in type 2 diabetes mellitus has a greater impact on physical health than depression and any other comorbid chronic disease (Moussavi et al., 2007). Those suffering from type 2 diabetes mellitus and depression are more likely to be on insulin treatment than oral agents or diet alone (Katon et al., 2004), and are also at increased risk of diabetic complications when compared to diabetes groups with other comorbid disease (de Groot et al., 2001). It is not clear whether this association between depression and diabetes complications is due to poorer glucose control in those with depression (elevated HbA_{1c}) or due to an

increase in inflammation in those with depression and comorbid type 2 diabetes mellitus, or both (Figure 3).

Treatments

It is recognised that depression in type 2 diabetes mellitus is far more common than the general population and is associated with worse health outcomes so both the American Diabetes Association (ADA) "Standards of Medical Care in Diabetes 2013" and the NICE guidelines alert diabetes physicians to the need to screen, identify and treat depression at the earliest stages of diabetes. (NICE, 2009; ADA, 2013). Once diagnosed, current treatments for depression in type 2 diabetes mellitus are similar to the treatments in the general population; however, in the UK some healthcare providers also have the provision of liaison psychiatry services. Psychiatrists in these services specialise in the treatment of patients with psychiatric symptoms who have been admitted to the hospital for other problems, for example, poor self-management of diabetes. Recent research suggests that these services are very successful at improving patient outcomes and are also more cost effective (NHS Confederation, 2011).

Controversies

Rates of depression have been found to be higher in studies where self-report measures are used so the increased prevalence of depression in type 2 diabetes mellitus may have been overstated (Anderson et al., 2001). Furthermore, many of the symptoms that self-report measures use overlap with diabetes symptoms (Krause et al., 2008; Richardson and Richards, 2008) and so thresholds used to define depression cases may need to be adjusted when used on type 2 diabetes populations (Twist et al., 2013).

Those with type 2 diabetes mellitus who receive treatment for comorbid depression are still an at-risk group for premature morbidity and complications

(Ismail et al., 2007). This suggests that there may be a pathogenesis of depression (such as increased systemic inflammation) that is not responsive to conventional treatments i.e. antidepressants or psychotherapy. It is possible that there might be several different aetiologies of depression that present with similar symptoms, which raises the question as to whether there is a higher proportion of depression with an inflammatory pathology in type 2 diabetes mellitus.

Conclusions

The research to date demonstrates that there is a bi-directional association between depression and type 2 diabetes mellitus and that those with both have a worse medical prognosis. There are a range of potential risk factors that have been suggested for both associations such as inflammation, differences in self-care behaviours and the burden of living with and managing type 2 diabetes mellitus. However, the studies to date have been predominantly cross-sectional and so the causal pathway is not yet clear. In particular, in people with newly diagnosed type 2 diabetes mellitus it is not clear if there is greater systemic inflammation in those with depression compared to those without depression, whether inflammation is a risk factor for the future development of depression or vice versa. This thesis examines the temporal relationship between depression and inflammation in a cohort of newly diagnosed type 2 diabetes mellitus patients. The main hypotheses are that: depression is associated with increased concentrations of inflammatory markers and is a risk factor for increased inflammation 12 months later; inflammation is a risk factor for the development of depression (or the change in depressive symptoms) 12 months later. If inflammation is a common risk factor for the development of depression and the poorer prognosis observed in this group, incident type 2 diabetes mellitus represents a window of opportunity to identify those patients at high risk of poor prognosis with the potential of early interventions.

Chapter 2: Methods

Synopsis

The methods used to test the hypotheses of this thesis are described in this chapter. A prospective cohort design was used to recruit a sample of the primary care population in three South London boroughs who had a recent (< 6 month) diagnosis of type 2 diabetes mellitus. The rationale for choosing this design, setting and sample population as well as all the explanatory variables and covariates measured are described here.

Aims and Objectives

There are four main aims of this thesis. Firstly, to examine whether, in a newly diagnosed type 2 diabetes mellitus population, there are cross-sectional correlations between systemically raised concentrations of inflammatory markers and depressive symptoms at baseline. To develop this further, the second aim is to identify any latent factors of inflammatory markers and examine their relationship with depressive symptoms. The third aim is to identify whether those with depression at baseline have increased systemic inflammation in the 12 months following diagnosis. Finally, the fourth aim of this thesis is to examine if there is a correlation between increased systemic inflammation at baseline and the onset of depression in the 12 months following diagnosis with type 2 diabetes mellitus.

Study Design

Cross-sectional study designs do not allow for causality or the temporal direction of the association between depression and inflammation in diabetes to be determined, therefore the study design used for this thesis is a prospective cohort design with cross-sectional data collection from patients at recruitment (within 6 months of diagnosis of type 2 diabetes mellitus) and at 12-month follow-up appointments.

A cohort study is an observational design where the study participants are recruited to examine the association between an initial explanatory (or independent) variable and an outcome (or dependent) variable. At baseline the explanatory variable of interest is measured, the participants are followed up for a period of time to test whether a specific outcome is more likely to be present in the group with the characteristic of interest (Woodward, 2013). There are two types of cohort studies, prospective and retrospective. This study is the former,

in which the outcome has not occurred when the study begins and the investigators follow-up the participants in the future (in this case 12 months). In the latter design, the outcome has already occurred when the study commences and data are collected retrospectively. While both study designs are accepted a prospective cohort has several advantages that make it ideal for this investigation.

Prospective cohort studies reduce the potential for selection bias. This occurs when the criteria for selection are associated with the outcome of interest and can lead to an overestimation of the relationship between the baseline characteristic and the outcome (Woodward, 2013). Furthermore, using a prospective cohort design, means any effects will be directly relevant to the population of patients in these South London boroughs and similarly to all patients within the UK - as the presence of a National Health Service (NHS) ensures medical practices are consistent across the nation. Although the sample sizes possible in a prospective cohort design are usually much smaller than those in retrospective cohort design, possibly the design's most limiting factor, it is the most appropriate for this investigation as many of the exposure variables of interest are not recorded in detail in routine care. The main limitation of the prospective study design are: that it is far more costly than the alternatives, due to the time needed to recruit, track and follow-up study participants; and losses to follow-up occur, these losses have the potential to introduce bias (discussed in Chapter 7) (Woodward, 2013).

Sample Description

Patients with type 2 diabetes mellitus were recruited within 6 months of diagnosis and were followed up for 24-months. Patients with newly diagnosed type 2 diabetes mellitus were selected as the study population, this represents a window of opportunity to identify patients at high risk of poor prognosis for which there is a potential for early intervention.

Although a newly diagnosed type 2 diabetes mellitus population was chosen as the study population, other populations could have been chosen to study the role of inflammation on depression. Diabetes complications are associated with depression (de Groot et al., 2001), however the presence of chronic pain neuropathy in populations with established type 2 diabetes mellitus (which is not often present in those who are newly diagnosed) (Winkley et al., 2013) and the prescription of low dose antidepressant medications as analgesics (BMA, 2010) would have been an additional source of confounding. Chronic pain would also have been a confounder when studying the association in other chronic disease populations (such as rheumatoid arthritis).

Inclusion Criteria

1. Adults (18-75) with a clinical diagnosis of type 2 diabetes mellitus as defined by the World Health Organisation's (WHO) criteria (WHO, 2006) within the last 6 months.

Exclusion Criteria

1. Non-English speakers, as the reliability of self-report psychiatric questionnaire may be reduced.
2. Temporary residents within the catchment area, as they may be more likely to be lost to follow-up.
3. Residents who reside primarily outside the catchment area, as they may be more likely to be lost to follow-up.
4. Patients with an end-stage condition, such as cancer, which may confound the association between the psychological variable and inflammation.
5. Patients with a severe mental illness recorded on their general practice notes: schizophrenia, dementia, bipolar (I or II) disorder, personality disorder or severe learning disabilities as the symptoms overlap depressive symptoms, particularly when measured by the PHQ-9.
6. Patients with severe diabetic complications, blind (secondary to diabetes), on kidney dialysis, or with an above knee amputation. These patients have likely been suffering from type 2 diabetes mellitus (without diagnosis) for some years and are not representative of someone who is early in the natural history of their diabetes.

Setting

The target population were primary care patients with newly diagnosed type 2 diabetes mellitus that resided within the South London boroughs of Lambeth, Southwark and Lewisham. Within this area all 138 general practices (primary care clinics in the UK's NHS) were invited to participate. There is a requirement for all general practices in the UK to set up and maintain an up-to-date diabetes register and so every 6 months patients with a first diagnosis of type 2 diabetes mellitus were identified from the electronic medical databases of participating general practices.

Recruitment

Researchers within the diabetes research group met with the practices that showed an interest to present the study and answer any further questions. Following this, participating surgeries allowed access to their respective electronic medical databases (EMIS LV, PCS & Web, Synergy and Vision) for screening of patients with a recent (no more than 6 months previously) diagnosis of type 2 diabetes mellitus. Patients' records were also screened for any exclusion criteria. The lead General Practitioner (GP) at each surgery then sent letters to patients that were potentially eligible for the study.

All eligible patients that were identified and wished to participate in the study were met by researchers at their GP surgery; here they gave written consent for participation in the study. The participants then completed a 'baseline' questionnaire and physical assessment and a fasting blood sample was collected. As collection of a fasting blood sample was not always possible, for example, if the appointment was in the afternoon or if the patient forgot to fast, the participants were given a blood form so that they could have a fasting blood test at their convenience in the following weeks. Twelve months after their baseline

interview, participants were seen for a follow-up appointment. Patients were seen for another follow-up appointment 24 months after baseline, but this data had not been collected at the time of writing this thesis.

Baseline Assessment

At baseline a one-off psychiatric interview (SCAN 2.1) was performed on all those participants that scored ≥ 10 on the PHQ-9 and on a random selection of 10% of the participants that scored < 10 on the PHQ-9. The purpose of this was to validate the use of the PHQ-9 in a diabetes population (Twist et al., 2013). The remainder of the procedure was identical at baseline and 12-month follow-up appointment.

At each visit a basic health check was conducted (height, weight, waist circumference, blood pressure) as well as a clinical examination of the feet. Venepuncture was also carried out during the appointment (where possible) for a blood sample for biological measures (see table 2.1). Finally, each subject was administered a self-report questionnaire.

Follow-Up Assessment

At 12 months after baseline, participants were contacted by letter to remind them of the follow-up appointments and were then contacted by telephone to schedule an appointment. To ensure that outcome data was collected, participants were contacted for a minimum 4-month window (beginning 1 month before the due date of their follow-up appointment). If after this time the patient was not contactable the data was coded as missing and available outcome data was collected from GP records.

Table 2.1: Data collected at baseline and 12-month follow-up appointments

Variable	Baseline	12-month follow-up
Age	YES	NO
Gender	YES	NO
Ethnicity	YES	YES
BMI	YES	YES
Smoking status	YES	YES
Macrovascular complications	YES	YES
PHQ-9	YES	YES
HbA1c	YES	YES
Lipids	YES	YES
CRP	YES	YES
IL-4	YES	NO
IL-6	YES	NO
IL-10	YES	NO
VEGF	YES	NO
TNF-α	YES	NO
IL-1β	YES	NO
IL-1RA	YES	NO
MCP-1	YES	NO
WBC	YES	NO
TGL	YES	YES
Adiponectin	YES	NO
Medications with anti-inflammatory action	YES	YES
Stressful life events	YES	YES

Measures

Main Explanatory Variables

The main explanatory variables included both biological and psychological variables; these are discussed in detail below.

Biological Variables

These variables were the inflammatory marker concentrations from serum, lipids, full blood count (for measurement of total white count) and HbA_{1c}.

Inflammatory Markers

Systemic inflammation was analysed through identification of inflammatory markers from whole blood samples taken after an overnight fast. All assays were carried out at GSTS King'sPath at King's College Hospital; assays for cytokines at the laboratory were conducted on serum samples that had been stored between -40 and 80°C. Twelve inflammatory markers that are associated *a priori* with type 2 diabetes and/or depression were selected to be tested (Pickup et al., 1997; Pradhan et al., 2001; Ridker et al., 2002; Pickup and Mattock, 2003; Pickup, 2004; Simon et al., 2008; Howren et al., 2009; Miller et al., 2009; Dowlati et al., 2010; Fernandez-Real and Pickup, 2012; Lamers et al., 2013).

i) C-reactive Protein

CRP is a 224-residue pentraxin protein. It was discovered in 1930 in patients infected with the Streptococcus pneumonia bacterium (Tillett and Francis, 1930) and is synthesised in the liver in response to macrophages and adipocytes as part of the acute phase response (Kindt et al., 2007). Its main mode of action is in cell apoptosis, where it binds to phosphocholine presented on the cell membrane of damaged cells (and some pathogens) and activates the complement system through macrophage phagocytosis (Ridker, 2009). CRP is readily detectable in blood where it rises from concentrations of < 1 mg/L to concentrations of > 200 mg/L during the acute phase response (Clyne and Olshaker, 1999). Due to its stability and ease of measure it is a commonly used screening tool for presence of inflammatory disease in the NHS where the reference range is 0-5 mg/L. CRP concentrations are considered a markers of cardiovascular disease risk concentrations of > 1 mg/L signify intermediate risk while concentrations > 3 mg/L are signify high risk (Pfutzner and Forst, 2006). Prophylactic use of statins in those with elevated CRP and without hyperlipidaemia significantly reduces the incidence of major cardiovascular events (Ridker et al., 2008).

The concentration of serum CRP was measured by a high-sensitivity CRP (hs-CRP) assay using an Advia 2400 analyser (Siemens Diagnostics, Frimley, UK) using reagents supplied by P.Z. Cormay. This assay was used instead of a standard CRP assay as it has a more sensitive detection limit of 0.1mg/L, which means it is possible to record definitive values for all study participants. Full methods can be found in the appendix.

CRP was reassessed at baseline and 12 months, CRP was chosen as the main measure of systemic inflammation as it is readily detectable and already used as a biomarker for inflammation in routine clinical care.

ii) Interleukins, Chemokines, Adipokines, Growth Factors and their Receptor Antagonists.

The concentrations of an array of endogenous inflammatory markers were measured using biochip kits (Randox, Belfast, UK). These biochip kits were analysed using the Randox Evidence Investigator (Randox). The inter- and intra-assay coefficients of variation for all analytes measured using these kits are <15% and <10% respectively. The markers of interest from these kits fell into three main categories, interleukins, adipokines and growth factors; a brief description of the main known effects of each marker follows although it should be noted that many of these markers are pleiotropic and so will have many actions not yet understood or described here.

In 1982 two laboratories simultaneously discovered a secreted protein that stimulated B cells, murine interleukin 4 (IL-4) (Howard et al., 1982; Isakson et al., 1982). Human IL-4 is a 153-residue protein and is understood to be secreted by basophils and T helper cells. IL-4 is also involved in T cell differentiation, the process by which naive T helper (Th0) cells are transformed to one of two classes of mature T helper (Th) cells, Th1 and Th2. IL-4 both inhibits differentiation of Th0 cells to Th1 cells and induced differentiation to Th2 cells, Th2 cells then produce and secrete more IL-4 in a feedback loop (Kindt et al., 2007).

IL-6 is a 212-residue adipokine protein, which induces the acute phase response. It was discovered by mistake by 1986 by Weissenbach et al. who were trying to clone interferon-beta (IFN-β) but accidentally cloned IL-6 mRNA, which they referred to at the time as IFN-β_2 (Vaquero et al., 1986). Human IL-6 binds to CD126 as part of the inflammatory response (Kindt et al., 2007) but has many effects one of which is the induction of endothelial production of MCP-1 (Romano et al., 1997). IL-6 has also been shown to increase permeability of (de Vries et al., 1996), and is capable of crossing, the blood brain barrier (Banks et al., 1994) and in the brain may be involved in the repression of BDNF (Patas et al., 2014).

The murine form of IL-10 was initially discovered by Fiorentino et al. and called cytokine synthesis inhibition factor (CSIF), as it inhibited the synthesis of cytokines (interferon-gamma (IFN-γ) and IL-2) by Th1 cells (Fiorentino et al., 1989). Human IL-10 is a 178-residue protein, produced by a variety of cells including T-cells, macrophages and mast cells. It is an anti-inflammatory cytokine and moderates inflammation through blockade of NF-κβ activity and inhibition of the synthesis of a number of cytokines, including IFN-γ and TNF-α (de Waal Malefyt et al., 1991). IL-10 is also an endogenous COX-2 inhibitor and may have a role in the inhibition of atherogenesis and vascular protection (Zemse et al., 2010; Sikka et al., 2013).

IL-1β, a 269-residue pro-protein, is one of the isoforms of IL-1 that bind to the IL-1 receptor and is secreted by damaged cells as well as macrophages (Kindt et al., 2007). The sequence of IL-1β was first reported in 1985 (March et al., 1985) although the IL-1 superfamily had been previously studied as 'the macrophage product' despite no amino-acid sequence being known. The protein is secreted in an inactive (pro-protein) form and is then proteolytically processed by the enzyme caspase 1 into its active form, which is involved in cell differentiation and apoptosis. IL-1β is also involved in the inflammatory response and fever (Kindt et al., 2007) and has been shown to increase permeability of the blood brain barrier (de Vries et al., 1996). It was not possible to measure the other isoform of IL-1 (IL-1α) from the serum samples as the molecule is not sufficiently stable and concentrations in the population were too low to measure reliably.

IL-1RA was discovered in the late 1980s by Arend and Dayer et al. (Arend and Dayer, 1990). Human IL-1RA is a 177-residue receptor antagonist that is expressed predominantly by epithelial cells. Receptor antagonists are ligands that bind competitively to receptors but do not provoke the biological response of the receptor agonist (Kindt et al., 2007). IL-1RA binds competitively to the IL-1 receptor, which is located on the cell surface, blocking IL-1 cytokine family.

Although by blocking pro-inflammatory IL-1 cytokines (such as IL-1β) the effects of IL-1RA are predominantly anti-inflammatory, increased concentrations of IL-1RA typically occur in response to increased inflammation as part of the acute phase response and so increased concentrations of IL-1RA indicate the presence of increased systemic inflammation (Arend, 2002).

Another adipokine studied is TNF-α, which was identified with TNF-β by Aggarwal et al. in 1984 (Aggarwal et al., 1984). TNF-α, a 233-residue protein, is particularly relevant to type 2 diabetes as it is secreted by macrophages in adipose tissue and stimulates the acute phase response through binding with the CD120 receptor (Kindt et al., 2007). MCP-1, a chemokine, is a small 99-residue protein that attracts inflammatory cells such as monocytes to sites of inflammation. Both TNF-α and MCP-1, like IL-1β and IL-6, have been implicated in increasing permeability of the blood brain barrier (de Vries et al., 1996; Stamatovic et al., 2005; Pan and Kastin, 2007).

Vascular endothelial growth factor (VEGF) was first isolated by Ferrara et al. in 1989 (Leung et al., 1989). VEGF is 232-residue protein that has chemotactic properties for macrophages and is involved in angiogenesis, vasculogenesis and endothelial cell growth. These processes are usually in response to cell hypoxia, which may be caused by exercise, injury or vessel blockage. VEGF is involved in the pathogenesis of diabetic retinopathy (Caldwelll et al., 2003; Caldwell et al., 2005) where it stimulates new vessel growth in response to retinal ischemia; it is likely that VEGF also plays a role in other microvascular complications of type 2 diabetes (Caldwelll et al., 2003; Caldwell et al., 2005).

These biomarkers were all measured from serum samples centrifuged from venous blood samples taken after an overnight fast and stored between -40 to -80°C, using cytokine-array biochip kits (Randox, Belfast, UK). These factors were

measured at baseline only, although future analysis of these biomarkers for the 12 and 24-month follow-up will be carried out for future investigation.

iii) Adiponectin

Murine adiponectin was first discovered in 1996 and was given its name as the mRNA was most highly expressed in adipocytes (Maeda et al., 1996). Human adiponectin is a 244-residue, 30 kilodalton (kDa) adipokine protein that is involved in glucose homeostasis and fatty acid breakdown and is negatively associated with body fat (Li et al., 2009). It has anti-inflammatory effects through the inhibition of NF-κβ signalling and regulation of the expression of TNF-α (Yamauchi et al., 2001). Adiponectin was measured using ELISA kits (R & D Systems Europe, Oxon, UK). The detection limit of the assay was 0.246 mg/L

iv) Triglycerides & High Density Lipoprotein

Triglycerides (TGL) and high density lipoprotein (HDL) are measures of dyslipidaemia and a proxy marker of inflammation. Dyslipidaemia occurs in response to inflammation and involves a series of changes to lipid metabolism in order to modulate the acute phase response (Esteve et al., 2005). Assays were carried out from blood serum samples using the Siemens Advia and Siemens Advia Direct HDL cholesterol method, a two-step automated procedure, using reagents provided by Siemens Healthcare Diagnostics Ltd. and a Siemens Advia 2400 analyser. Full methods can be found in the appendix. The detection limits of the assays were: triglyceride 0.01 mmol/L, total cholesterol 0.01 mmol/L, HDL-cholesterol 0.1 mmol/L; LDL-cholesterol was calculated via the Friedewald formula.

v) Glycated Haemoglobin

HbA_{1c} was measured at baseline and 12-month follow-up. This was because blood sugars are likely to fluctuate during the course of the study. HbA_{1c} is

relevant as both an outcome measure for management of diabetes and also as a confounder (as there is a close relationship between high blood sugars and inflammation). Assays are conducted by affinity chromatography from whole blood taken with the anticoagulant EDTA using the Primus Ultra 2 analyser (Primus Corporation, Kansas City, US). Full methods can be found in the appendix.

HbA_{1c} is a proxy marker of glycaemic control over a 12-week period, although it is more closely related to glycaemic control over a 6-week period due to the lifespan of red blood cells; these have no nucleus and live for 12 weeks (Florkowski, 2013). Sugar attaches itself to haemoglobin on red blood cells at an increasing rate depending on the concentration of sugar in the blood. The HbA_{1c} measures the amount of glucose attached to the N terminal of the B chain of haemoglobin molecules. The reference range for people with normal glucose homeostasis is 4.5-6.5% (Holt et al., 2011). The aim of diabetes treatment is to achieve an HbA_{1c} of < 7% without any hypoglycaemic events. As very intensive interventions are required to achieve an HbA_{1c} within the normal range, the UK NICE guidelines advise a target HbA_{1c} for the diabetes population of 6.5% (NICE, 2008). Higher HbA_{1c} is correlated to higher concentrations of inflammatory markers. This may be through AGE stimulated inflammatory processes or increased HPA axis stress response. Throughout this thesis HbA_{1c} is reported in both IFCC and DCCT units as has been recommended by the international HbA_{1c} consensus committee and is commonplace in leading diabetes journals (Hanas and John, 2013).

vi) Full Blood Count for the Measure of Total White Count

Total white blood cell count (WBC) or leukocyte count is a measure of the immune cells (neutrophils, lymphocytes, eosinophils, basophils and monocytes) from whole blood, these cells all mature from hematopoietic stem cells in bone marrow (Kindt et al., 2007). Leucocytosis, a WBC above the normal range in

blood is an indicator of an inflammatory response, but also occurs after exercise and psychosocial stress (Rogers, 2010).

FBC assays are conducted from whole blood taken with the anticoagulant EDTA using the Siemens Advia 2120 using reagents provided by Siemens Healthcare Diagnostics Ltd. WBC was measured using an Advia 2120 analyser (Siemens Diagnostics); these tests were carried out using freshly drawn venous blood samples stored at room temperature until analysed. Full methods can be found in the appendix.

Psychological Variables

Antidepressant medications were recorded in the 'other' section of the data collection schedules at each visit. However, at commencement of this study it was decided that this was not detailed enough information and so a new data collection schedule was developed. This was administered at the time of the 24-month follow-up appointment (or collected from GP records at that time for those participants who did not complete the final follow-up visit) and so was not available for use in my thesis. This records information on the prescription of antidepressants in the 36 months before the 24-month follow-up appointment. Information recorded was: whether the minimum therapeutic dose for depression was prescribed of each respective medication, the dates that the medication was prescribed between, whether there were any gaps in this period of prescribing that were greater than 6 months.

i) Depression

Depression was assessed using the self-report PHQ-9 (self-report tools are commonly used to identify cases of depression and depressive symptoms in large epidemiological studies). The PHQ-9 is a nine-question screening questionnaire for depression that scores nine diagnostic criteria for depression laid out by the DSM-IV (APA, 2000). Questions are graded on a four-point scale of zero to three, where zero is not at all and three is almost every day. The PHQ-9 is particularly useful as it allows for both a continuous and categorical outcome. From the questions a score within the range of 0-27 is produced, indicating the level of depressive symptoms, where a score of ≥ 10 is an indicator of depression. Use of self-report questionnaires has been shown to be adequate for research of this nature by a recent meta-analysis, and the use of full diagnostic criteria in comparison has shown similar results (Nouwen et al., 2010).

The main outcome is the difference in the rate of depression between participants with a lower level of circulating inflammatory markers and a higher level of circulating inflammatory markers.

Use of PHQ-9 for identifying cases of depression was validated by measuring depression at baseline using the WHO Schedule for Clinical Assessment in Neuropsychiatry 2.1 (SCAN 2.1) (Wing et al., 1998). This is a semi-structured comprehensive diagnostic interview which non-clinicians can be trained to administer, as the inter-rater reliability between scan 2.1 trained interviewers and clinicians is high (Brugha et al., 1999).

As there is some evidence to suggest that a higher cut-off of the PHQ-9 has greater validity than the cut-off of ≥ 10, (Reddy et al., 2010; van Steenbergen-Weijenburg et al., 2010) a receiver operating characteristic (ROC) curve was used to test the validity of the PHQ-9 as a screening tool in this population of newly diagnosed type 2 diabetes mellitus. The ROC curve plots specificity (the

true-positive rate of the test) against sensitivity (the true-negative rate of the test) for each possible cut-off point of the PHQ-9 score. The Youden index measures the performance of diagnostic tests, equally accounting for false positives and false negatives (Youden, 1950), and was used to determine an optimal cut-off of the PHQ-9 score in this diabetes population. Using this index a cut-off of ≥ 12 was determined as the optimal cut-off for depression, however use of this cut-off comes at the cost of a reduced sensitivity of 87% (compared to a sensitivity of 95% at the PHQ-9 cut-off of ≥ 10) (Twist et al., 2013). Use of the PHQ-9 cut-off of ≥ 12 compared to the cut-off of ≥ 10 is discussed in Chapter Three, where both cut-off are used to compare depression cases to non-depressed subjects. The cut-off of ≥ 10 is used as an indicator of probable depression for the remainder of the thesis.

Covariates

The covariates included socio-demographic and biological factors.

Socio-Demographic Variables

i) Age & Gender

Age and gender were recorded from GP electronic healthcare records (EMIS LV, EMIS PCS, EMIS Web, Synergy and Vision systems). For the purpose of analyses age was converted to a decimal value in years. Gender was coded as either male or female.

ii) Ethnicity

Ethnicity was measured using a self-report questionnaire based on UK census methods (HMSO, 2001). For the purposes of adjusted analyses in this thesis ethnicities were categorised as two groups, those of White ethnic origin and those of Black/Mixed ethnic origin.

iii) Stressful Life Events

Stressful life events were recorded at 12-month follow-up by self-report questionnaire based on the PERI Life Events Scale, a list of 102 potential stressful life events relevant to an urban setting (Dohrenwend et al., 1978). Questions are worded so that whether the life event was perceived as stressful did not influence how each question was answered. As evidence suggests increased depression with any psychosocial stress, stressful life events were classified as none, or more than 1. Adversity and stress have recognised effects on the HPA axis, inflammation and depression so this was an important covariate to measure when investigating longitudinal associations.

Biological Variables

i) Body Mass Index

BMI was calculated from weight and height of the participants, the researchers measured these variables at each visit using calibrated scales and height charts.

ii) History of Macrovascular Disease

History of macrovascular disease was recorded from GP electronic healthcare records (EMIS LV, EMIS PCS, EMIS Web, Synergy and Vision systems). Participants were coded as having had any of the following macrovascular events: myocardial infarction (MI), coronary artery bypass graft (CABG),

cardiovascular accident (CVA), coronary angioplasty, limb revascularisation.

Treatments

Treatments were measured from GP electronic healthcare records. Data were collected for all acute and repeat prescriptions the patient was receiving at the time of each interview. As is the case with large cohort studies, it was not possible to measure adherence to medication. Prescribed medications were recorded from GP electronic healthcare records and patients not adhering to medications will likely have not requested repeat prescriptions so the least adherent patients will not have been recorded as receiving treatment.

Diabetes treatments were recorded in the same way as other medications; however, as detailed information on insulin therapy dose is not usually included on GP electronic healthcare records, insulin was collected using a self-report questionnaire from those participants that were receiving insulin.

Complications

The presence of complications was recorded from GP electronic healthcare records. Macrovascular complications recorded at follow-up were the same categories as those at baseline. Macrovascular complications recorded were: retinopathy, foot ulcers and amputations.

Comorbid Conditions

Any long-term comorbid conditions were recorded from GP electronic healthcare records so that these could be adjusted for; unfortunately, this data was not available for this thesis.

Ethical Approval

Ethical approval was sought and granted for all methods and data collection, by the King's College Hospital Research Ethics Committee (reference 08/H0808/1) and by Lambeth, Southwark and Lewisham Primary Care Trusts (reference RDLSLB 410) and all participants gave written informed consent. Additional minor amendments to include ethical approval for several additions to the questionnaires, such as the comorbidity table, was sought and approved. This meant that all data on comorbid diseases, which may have an impact on results (such as cancers and other diseases causing an acute phase response), could be collected from participants' GP surgery records.

Maximising Outcome Data

As this study formed a small part of a large cohort study, it was not possible to ensure participants were interacting with the same researcher at each follow-up visit. Additionally, as not all participants were able to have blood tests at the time of interview, it was noticed at baseline that this was the biggest source of missing data. Several precautions had been taken before commencement of the study in order to maximise outcome data. Firstly, at recruitment temporary residents were excluded from the study and secondly the research team emphasised to the participants that the study was for 2 years and involved three appointments; participants were informed about the time these would take. These practices helped to reduce the number of people recruited that would then withdraw from follow-up or become non-contactable.

After it was noted that blood data was the biggest source of missing data at baseline the data manager created monthly lists of those participants for whom

we had not received blood test results and the researchers routinely contacted these participants to remind them, offer to send out new blood packs or arrange an appointment for venepuncture. These practices reduced the missing data for blood tests (from those participants who attended an appointment) from approximately 30% at baseline to approximately 10% at 24-month follow-up.

Sample Size & Power Calculation

The required power for the longitudinal analyses in Chapter 5 was calculated to 80% of achieving a significant result at the 5% level. For the first analysis the estimated standardised mean difference of interleukins was d = 0.35 greater in depressed than in non-depressed subjects with diabetes based on a recent meta-analysis (Howren et al., 2009). With a type 1 error rate of 5%, 80% power and depressed: non-depressed ratio of 1:7, n = 74 and n = 518 participants will be needed respectively. Assuming an over-cautious 15% attrition rate, the required sample size is *N* = 697 participants.

For the longitudinal analyses in Chapter 6, an incidence of depression of 10 patients per 1000 per year in the general population (1% of the population after 1 year, 2 % of the population after 2 years) and a reported incidence of depression, when diagnosed through self-report questionnaires, of up to 2 to 3 times that of the general population in patients who suffer from treated type 2 diabetes mellitus (Golden, 2007) (2.5% of the population after 1 year, 5% of the population after 2 years) was assumed. If the hypothesis is correct and the increase in depression previously identified in diabetic populations (Polsky, 2005; Engum, 2007; Maraldi, 2007) is due to higher grade systemic inflammation, the group with lower inflammation should have an incidence close to the normal non-diabetic incidence of depression and those with raised systemic inflammation should have an incidence typically higher and more representative of a population of those with treated diabetes. A minimum sample

size of > 1199 participants per group was necessary for adequate power with depression used as a categorical variable at 12-month follow-up, this decreases to > 588 per group at 24-month follow-up.

Statistics

Data were analysed using SPSS 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics, Version 21.0. Armonk, NY). The main characteristics of the study population throughout this thesis are summarised as mean (standard deviation [SD]) where data were normally distributed, median (interquartile range [IQR]) where data were skewed, or as a count (percentage) for categorical variables. Unadjusted statistical analyses were conducted using Student's *t* test for normally distributed continuous data, Mann-Whitney U analyses for non-normally distributed continuous data and Spearman's ranked correlation coefficient (r_s) to compare bivariate associations. The chi-squared (χ^2) test was used for comparisons of categorical data. A natural log was used to transform skewed data for inflammatory markers and PHQ-9 score in multiple regressions. Multiple linear regressions were used to assess the relationship between depressive symptom score and those inflammatory markers that had significant association in unadjusted analyses; covariates were added to the models in sequential steps using a hierarchical method and only retained if they were significantly associated with the outcome or an important clinical confounder, e.g. ethnicity. Simes' improved Bonferroni method was used to correct for multiple testing for multiple comparisons of inflammatory marker differences between groups and association between inflammatory marker concentrations and PHQ-9 score, to reduce the risk of a type 1 error. A natural log was used to transform skewed data in multiple regressions and the factor analysis. Exploratory maximum likelihood factor analysis was used derive the factor structure of the PHQ-9 depressive symptom score and inflammatory markers.

Discussion

The aims of this thesis are to examine whether, in a newly diagnosed type 2 diabetes mellitus population, there is a cross-sectional correlation between systemically raised concentrations of inflammatory markers and depressive symptoms at baseline and 12 months later. Secondly, to identify any latent factors of inflammatory markers – in order to examine the relationship between any composite measure of inflammation and depressive symptoms. Thirdly, to examine if there is a correlation between increased systemic inflammation at baseline and the onset of depression in the 12 months following diagnosis with type 2 diabetes mellitus. Finally, to identify whether those with depression at baseline have increased systemic inflammation in the 12 months following diagnosis with type 2 diabetes mellitus.

A prospective cohort design was chosen as this will allow the measurement of exposure characteristics and outcome variables such as PHQ-9 depressive symptom score and inflammatory markers concentrations not used in routine clinical practice. This allows for longitudinal associations between explanatory variables and outcomes to be determined while adjusting for the effects of covariates.

Not all questionnaire and biological variables measured in this cohort are described here, as they are not relevant to this thesis. Additionally, it was not possible to measure some variables, which may be of interest to the associations studied in this thesis, notably diet and physical activity. In a large epidemiological study it is not feasible to collect objective data on these variables without incurring extra costs for equipment such as pedometers, (and the administration required to distribute and collect them) and due to additional participant attrition.

The next chapter describes the baseline characteristics of a cohort of people with newly diagnosed type 2 diabetes mellitus, stratified by depression status.

Chapter 3: The Cross-sectional Association of Depression and Inflammatory Markers

Synopsis

This aim of this chapter is to examine the cross-sectional relationship between depressive symptom score and an array of inflammatory markers at baseline in a newly diagnosed type 2 diabetes mellitus population. The hypothesis that higher depressive symptom scores are associated with higher concentrations of inflammatory markers was tested. The independent association between inflammatory markers and depressive symptom score was estimated by multiple linear regression, covariates adjusted for included, socio-demographic factors, adiposity, macrovascular disease, smoking, HbA_{1c} and prescribed medication. A secondary hypothesis tested was that concentrations of inflammatory markers were higher in those with probable depression, determined by the PHQ-9, and validated by the SCAN 2.1. Two between-group comparisons were made between depression cases and non-depressed subjects. Classification of depression was based on two cut-offs of the PHQ-9 score that have validity in general population and in this sample of type 2 diabetes mellitus patients respectively. The main results were, depressive symptom score was associated with eight inflammatory markers and this association remained for five inflammatory markers after adjusting for covariates. The main conclusions are that there is a cross-sectional association between five inflammatory markers and depressive symptom score. It is not possible to determine which direction of the association between depression and type 2 diabetes mellitus inflammation may be involved in because no longitudinal associations were studied.

Introduction

As is described in Chapter 1, the prevalence of depression and depressive symptoms is at least twice as high in individuals with type 2 diabetes mellitus compared to those without diabetes (Anderson et al., 2001; Nichols and Brown, 2003). When depression and depressive symptoms are present, they are associated with worse glycaemic control (Lustman et al., 2000), an increased risk of microvascular and macrovascular diabetes complications (de Groot et al., 2001) and an increased risk of premature mortality (Katon et al., 2005). In prospective studies, the presence of depressive symptoms are associated with a 37%-60% increased risk of incident type 2 diabetes mellitus, and there is a smaller reverse association of a 15%-24% risk of incident depressive symptoms in those with type 2 diabetes mellitus (Mezuk et al., 2008; Nouwen et al., 2010).

The mechanisms for the bi-directional relationship between depressive symptoms and diabetes are not well understood (Ismail, 2010; Renn et al., 2011). This thesis is investigating activated innate immunity as the common antecedent for the parallel development of type 2 diabetes mellitus (caused by insulin resistance and beta cell failure or impaired insulin secretion) and depression, since there is evidence for the involvement of innate immunity in the pathogenesis of both.

The literature review in Chapter 1 explained the proposed mechanisms for inflammation in the pathogenesis of depression and type 2 diabetes mellitus which are supported by separate research on inflammation in depression alone, depression in other chronic conditions and in type 2 diabetes mellitus. In addition, two previous studies, with small sample sizes with different durations of type 2 diabetes mellitus, identified an association between inflammatory markers and depressive symptoms (Zeugmann et al., 2010) (Doyle et al., 2013), though when this study was initiated neither of these two studies had been published. As these populations were of established diabetes and an inpatient

sample, these associations are likely subject to confounding due to late diabetes complications and insulin treatment – both are known to be increased in depression.

Despite these proposed mechanisms, and the associations identified in established type 2 diabetes mellitus, in people with newly diagnosed type 2 diabetes mellitus it was not known if there is a cross-sectional association between systemic inflammation and depressive symptoms. In this chapter the hypothesis that the presence of depressive symptoms are associated with increased concentrations of circulating inflammatory markers in a cohort of newly diagnosed type 2 diabetes mellitus patients is tested. Newly diagnosed cases of type 2 diabetes mellitus were selected as this period represents a window of opportunity to identify patients at high risk of poor prognosis with the potential of early intervention.

Methods

Design, Setting, Sampling frame: The study design, setting and sampling frame were for the baseline participants in the SOULD study and have been previously described in the methods chapter (Chapter 2).

Study population and case definition: People with a first (< 6 months) diagnosis of type 2 diabetes mellitus according to the WHO's criteria (WHO, 2006), aged between 18 and 75 years at diagnosis, were identified from diabetes registers of participating general practices and invited to participate. Recruitment and baseline appointments for data presented in this chapter were conducted between May 2008 and September 2012; the sampling methodology is described in further detail in Chapter 2.

Main explanatory variables: Twelve inflammatory markers were selected which had been identified *a priori* as associated with type 2 diabetes mellitus and/or depression (Pickup et al., 1997; Ridker et al., 2002; Pickup and Mattock, 2003; Pickup, 2004; Kadowaki et al., 2006; Leo et al., 2006; Simon et al., 2008; Dowlati et al., 2010; Fernandez-Real and Pickup, 2012; Duivis et al., 2013; Lamers et al., 2013). Serum CRP was measured by an hs-CRP assay using freshly drawn venous blood samples stored at room temperature until analysed. Adiponectin was measured using ELISA kits and an array of inflammatory markers (IL-4, IL-6, IL-10, VEGF, TNF-α, IL-1β, IL-1RA, and MCP-1) were measured using cytokine-array biochip kits. The assays were carried out from serum samples centrifuged from venous blood samples which were taken after an overnight fast and stored between -40 to -80°C. Fasting lipids and WBC were measured using a Siemens Advia 2400 analyser, LDL-cholesterol was calculated via the Friedewald formula. Fasting lipid tests and WBC assays were carried out using freshly drawn venous blood samples stored at room temperature until analysed. Fasting blood samples were drawn in the morning at the participants' respective general practices or their local phlebotomy service and were centrifuged, analysed, aliquoted and

then frozen (for the cytokine analyses) on the same day. Detailed methods and a description of each of these variables are provided in full in the methods chapter (Chapter 2).

Main outcome variable: The presence of depressive symptoms at baseline was measured using the PHQ-9. This is a self-report measure that was developed for primary care to aid clinicians in identifying probable cases of depression. A cut-off score of ≥ 10 is the optimal threshold for identifying probable cases of depression, with a sensitivity of 73% and specificity of 98 % (Spitzer et al., 1999). PHQ-9 scores ≥ 5, 10, 15, and 20 represented mild, moderate, moderately severe, and severe depression respectively (Kroenke et al., 2001). The PHQ-9 also is valid in diabetes populations where a cut-off score of ≥ 12 may be more appropriate for identifying probable cases of depression (Twist et al., 2013). Full details on the collection of this data are presented in Chapter 2.

Assessment of confounders: The following data were measured and coded using general practice records: age, gender, history of macrovascular disease (MI, CABG, CVA, and carotid or limb re-vascularisation), current prescribed medications with a possible anti-inflammatory action (statins, fibrates, systemic steroids, non-steroidal anti-inflammatory drugs, COX-2 inhibitors). HbA_{1c} was measured by affinity chromatography using freshly drawn venous blood samples stored at room temperature until analysed. A physical examination was performed to measure systolic and diastolic blood pressures (mmHg) and body mass index (kg/m^2) and a questionnaire was used to determine smoking status and self-report ethnicity based on 2001 UK Census methods. Detailed information on each variable and of their relevance as confounders has been previously described in Chapter 2.

Statistical Analyses: Data were analysed using SPSS 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics, Version 21.0. Armonk, NY). The main characteristics of

the study population are summarised as mean (SD) where data were normally distributed, median (IQR) where data were skewed, or as a count (percentage) for categorical variables, and are stratified by PHQ-9 depression case status. Unadjusted statistical analyses were conducted using Student's *t* test for normally distributed continuous data, Mann-Whitney U analyses for non-normally distributed continuous data and r_s to compare bivariate associations. The χ^2 test was used for comparisons of categorical data. A natural log was used to transform skewed data for inflammatory markers and PHQ-9 score in multiple regressions.

Multiple linear regressions were used to assess the relationship between depressive symptom score, as the dependent variable and those inflammatory markers that had significant association in the unadjusted analyses as independent variables; covariates were added to the model in sequential steps using a hierarchical method and only retained if they were significantly associated with the outcome or an important clinical confounder, such as ethnicity. An assessment of the residuals did not suggest major violations of the assumptions of a multiple regression.

Left-censored data for concentrations of inflammatory markers (where the actual concentration was below the minimum detection threshold) accounted for less than 2% of values. Left-censored values were replaced with values equal to half the minimum detected value for each cytokine. There were five subjects with right-censored values for concentrations of MCP-1 (where the concentration was above the maximum detection threshold), as the bivariate analyses used ranked data and medians these were included as maximum values. As right-censored values accounted for less than 0.5% of the total MCP-1 values and a sensitivity analysis showed these values no effect on the results of the regression, subjects with right censored data were omitted from the adjusted multiple linear regression analyses.

An alpha (significance) level of < 0.05 was chosen as sufficient to reject the null hypothesis of any statistical test, at this level there is a 5% (1/20) chance of a type 1 error in any test. As there are multiple inflammatory measures in this chapter which are being investigated with respect to PHQ-9 score, the chance of a type 1 error is increased. To correct for the increased risk in type 1 error, Simes' improved Bonferroni method was used to correct for multiple testing for pair-wise comparisons of inflammatory marker differences between groups and association between inflammatory marker concentrations and PHQ-9 score.

Results

Of 139 general practices invited, 96 (70%) agreed to participate, from which 1790 participants with newly diagnosed type 2 diabetes mellitus were recruited. There were > 25% missing answers from PHQ-9 scores in 21 (1.2%) participants, so these were excluded and the analyses were conducted on n = 1769 (98.8%). Analyses of IL-4, IL-6, IL-10, VEGF, TNF-α, IL-1β, IL-1RA and MCP-1 were conducted on a subset of the cohort for whom a serum sample had been stored, n = 1227 (69%). Compared to those with a frozen stored sample, those who had missing or un-analysable blood samples (n = 542) were younger (mean age 57 (11) vs. 55 (11) years, $p < 0.001$) and more likely to be of Black African or Caribbean ethnicity (36% vs. 50%, $p < 0.001$) but there were no statistically significant differences in gender (55% vs. 56% male, $p = 0.838$), PHQ-9 depression cases (13.6% vs. 17.8%, $p = 0.08$) or glycaemic control (DCCT % HbA_{1c} 6.97 (1.4) vs. 7.09 (1.6) (IFCC HbA_{1c} 52.7 mmol/mol (15.2) vs. 54.0 mmol/mol (17.3)), $p = 0.148$). The median age for female participants was 57 (IQR 50-64) years, suggesting that the majority were probably post-menopausal. Analyses of CRP were conducted on the whole cohort for whom concentration of CRP was measurable at baseline, n = 1461 (82.5%) (Table 3.2).

Table 3.1: Baseline characteristics of participants with type 2 diabetes mellitus in the South London Diabetes Study stratified by whether there was a frozen serum sample for cytokine array for subjects

Baseline variable	Total (n = 1769)	Serum Sample (n = 1227)	No Serum Sample (n = 542)	p-value
Mean age, years	56.1 (11.04)	56.7 (10.99)	54.8 (11.01)	0.001*
Gender (%)				
Male	976 (55.2)	675 (55.0)	301 (55.5)	0.838
Female	793 (44.8)	552 (45.0)	241 (44.5)	
Ethnicity (%)				
White	878 (49.6)	660 (53.8)	218 (40.2)	<0.001*
Black	710 (40.1)	439 (35.8)	271 (50.0)	
Asian/Other	181 (10.2)	128 (10.4)	53 (9.8)	
HbA_{1c}				
Mean % HbA_{1c}	7.00 (1.45)	6.97 (1.40)	7.09 (1.59)	0.148
Mean HbA_{1c}, mmol/mol	53.1 (15.9)	52.7 (15.3)	54.0 (17.4)	
Psychological assessment				
Median PHQ-9 score	3.0 (0.0-6.0)	2.0 (0.0-7.0)	3.0 (0.0-6.0)	0.042*
PHQ-9 depression (%)	258 (14.6)	167 (13.6)	91 (16.8)	0.081

***significant α=0.05. T-test and Mann Witney U test used for normal and non-normal data**

Table 3.2: Baseline characteristics of participants with type 2 diabetes mellitus in the South London Diabetes Study stratified by whether there was a serum sample (for CRP analysis) for subjects

Baseline variable	Total (n = 1769)	Serum Sample (n = 1461)	No Serum Sample (n = 308)	p-value
Mean age, years	56.1 (11.04)	56.4 (10.86)	54.5 (11.72)	0.006*
Gender (%)				
Male	976 (55.2)	797 (54.6)	179 (58.1)	0.253
Female	793 (44.8)	664 (45.4)	129 (41.9)	
Ethnicity (%)				
White	878 (49.6)	738 (50.5)	140 (45.5)	0.144
Black	710 (40.1)	571 (39.1)	139 (45.1)	
Asian/Other	181 (10.2)	152 (10.4)	29 (9.4)	
HbA_{1c}				
Mean % HbA_{1c}	7.00 (1.45)	6.95 (1.37)	7.36 (1.89)	0.002*
Mean HbA_{1c}, mmol/mol	53.1 (15.9)	52.5 (14.9)	56.9 (20.7)	
Psychological assessment				
Median PHQ-9 score	3.0 (0.0-6.0)	3.0 (0.0-6.0)	2.6 (0.0-6.0)	0.789
PHQ-9 depression (%)	258 (14.6)	212 (14.5)	46 (14.9)	0.848

***significant α=0.05. T-test and Mann Whitney U test used for normal and non-normal data**

Table 3.3a: Baseline characteristics of depression cases (PHQ-9 ≥ 10) and non-depressed (PHQ-9 < 10) subjects with type 2 diabetes mellitus in the South London Diabetes Study

Baseline variable	Total (n = 1769)	No depression (n = 1511)	Depression (n = 258)	p-value
Mean age, years	56.1 (11.04)	56.6 (11.05)	53.0 (10.40)	<0.001*
Gender (%)				
Male	976 (55.2)	855 (56.6)	121 (46.9)	0.004*
Female	793 (44.8)	656 (43.4)	137 (53.1)	
Ethnicity (%)				
White	878 (49.6)	750 (49.6)	128 (49.6)	0.128
Black	710 (40.1)	615 (40.7)	95 (36.8)	
Asian/Other	181 (10.2)	146 (9.7)	35 (13.6)	
Mean % HbA_{1c} (Mean HbA_{1c}, mmol/mol)	7.00 (1.45) (53.1)	6.98 (1.46) (52.8)	7.13 (1.43) (54.5)	0.139
Lipids				
Median triglyceride, mmol/L	1.40 (0.90-2.00)	1.40 (0.90-1.90)	1.50 (1.00-2.20)	0.003†
Mean low density lipoprotein, mmol/L	2.6 (0.91)	2.63 (0.90)	2.65 (0.98)	0.792
Mean high density lipoprotein, mmol/L	1.2 (0.34)	1.22 (0.33)	1.20 (0.39)	0.589
Mean total cholesterol, mmol/L	4.6 (1.09)	4.56 (1.06)	4.69 (1.23)	0.097
Mean body mass index, kg/m^2	32.0 (6.50)	31.8 (6.34)	33.4 (7.23)	0.001*
Macrovascular disease (%)				
None	1584 (90.8)	1365 (91.5)	219 (86.2)	0.007*
More than 1	161 (9.2)	126 (8.5)	35 (13.8)	
Smoking Status (%)				
Smoker	353 (21)	271 (18.8)	82 (34.3)	<0.001*
Non-Smoker	1324 (79)	1167 (81.2)	157 (65.7)	
Inflammatory markers				
Median CRP, mg/L	2.90 (1.20-6.40)	2.70 (1.10-6.20)	3.25 (1.40-8.70)	0.002†
Median IL-4, ng/L	1.34 (1.11-1.68)	1.34 (1.10-1.68)	1.35 (1.17-1.68)	0.380
Median IL-6, ng/L	1.35 (0.68-3.42)	1.32 (0.67-3.41)	1.40 (0.75-3.63)	0.420
Median IL-10, ng/L	0.45 (0.34-0.63)	0.44 (0.34-0.63)	0.47 (0.35-0.68)	0.144
Median VEGF ,ng/L	75.3 (44.8-119.3)	74.6 (44.9-118.2)	83.29 (44.9-128.7)	0.589
Median TNF-α, ng/L	0.89 (0.39-1.86)	0.89 (0.39-1.88)	0.91 (0.43-1.76)	0.771
Median IL-1β, ng/L	1.02 (0.73-1.87)	1.01 (0.72-1.85)	1.12 (0.76-2.06)	0.191
Median IL-1RA, ng/L	437 (292.0-694.8)	430.0 (282.9-672.4)	488.8 (337.3-785.3)	0.003†
Median MCP-1, ng/L	103.3 (60.2-152.9)	101.7 (59.3-151.1)	112.1 (62.8-162.6)	0.134
Median WBC, $x10^9/L$	6.51 (5.31-7.97)	6.44 (5.25-7.86)	6.91 (5.74-8.56)	<0.001†
Median adiponectin, mg/L	4.94 (3.28-7.50)	5.01 (3.29-7.61)	4.65 (3.27-6.65)	0.097

***significant α=0.05. †significant after Simes' improved Bonferroni correction for multiple testing. Missing or incomplete values for: HbA_{1c} = 117 cases, lipids = 245 cases, BMI = 3 cases, macrovascular history = 24 cases, smoking status = 92 cases, CRP = 308 cases, WBC = 183 cases, adiponectin = 304 cases. Values for IL-4, IL-6, IL-10, VEGF, TNF-α, IL-1β, IL-1RA, MCP-1 were for a subset of first 1227 subjects for whom there was a stored serum sample.**

Table 3.3a reports the demographic characteristics, depressive symptom score and concentrations of inflammatory markers in the type 2 diabetes mellitus patients stratified by PHQ-9 depression case status. The prevalence of depression cases, defined as a PHQ-9 score ≥ 10, was 14.6% (n = 258). Depression cases were nearly 5 years younger, had a higher BMI, were more likely to be female, were more likely to smoke and had a significantly greater prevalence of macrovascular disease. Table 3.3b reports the same as Table 3.3a, but stratified by PHQ-9 depression status using a cut-off of ≥ 12. The prevalence of depression cases using the cut-off score of ≥ 12 was 11.2% (n = 198) was significantly lower than when the cut-off ≥ 10 was used ($p < 0.05$).

Median circulating concentrations of the inflammatory markers CRP, IL-1RA, WBC and TGL were significantly higher in depression cases compared to non-depressed subjects and these differences remained significant after Simes' improved Bonferroni correction for multiple testing (Table 3.3a). There were no statistically significant differences in LDL-cholesterol, HDL-cholesterol, total cholesterol, IL-4, IL-6, IL-10, VEGF, TNF-α, IL-1β, MCP-1 or adiponectin concentrations in depression cases compared to non-depressed subjects, although there was a trend towards lower concentrations of adiponectin in the former group. In Table 3.3b the reported results are similar to Table 3.3a with the exception of TGL, which is identical between depression cases versus non-depressed subjects when using the cut-off ≥ 12.

Symptoms of depression, measured as a continuous PHQ-9 score, were positively correlated with the inflammatory markers CRP, VEGF, IL-1β, IL-1RA, MCP-1, WBC, and TGL and were negatively correlated with adiponectin; these differences remained significant after Simes' improved Bonferroni correction for multiple testing (Table 3.4). The mean HbA_{1c} concentration was not statistically different between depression cases and non-depressed subjects.

Table 3.3b: Baseline characteristics of depression cases (PHQ-9 ≥ 12) and non-depressed (PHQ-9 < 12) subjects with type 2 diabetes mellitus in the South London Diabetes Study

Baseline variable	Total (n = 1769)	No depression (n = 1571)	Depression (n = 198)	p-value
Mean age, years	56.1 (11.04)	56.5 (11.03)	52.6 (10.43)	<0.001*
Gender (%)				
Male	976 (55.2)	884 (56.3)	92 (46.5)	0.009*
Female	793 (44.8)	687 (43.7)	106 (53.5)	
Ethnicity (%)				
White	878 (49.6)	783 (49.8)	95 (48.0)	0.050
Black	710 (40.1)	637 (40.5)	73 (36.9)	
Asian/Other	181 (10.2)	151 (9.6)	30 (15.2)	
Mean % HbA_{1c}	7.00 (1.45)	6.99 (1.46)	7.15 (1.42)	0.150
Mean HbA_{1c}, mmol/mol	53.1 (15.88)	52.9 (15.92)	54.6 (15.50)	
Lipids				
Median triglyceride, mmol/L	1.40 (0.90-2.00)	1.40 (0.90-1.90)	1.40 (1.00-2.10)	0.053
Mean low density lipoprotein, mmol/L	2.63 (0.91)	2.63 (0.91)	2.63 (0.94)	0.961
Mean high density lipoprotein, mmol/L	1.22 (0.34)	1.22 (0.33)	1.18 (0.35)	0.126
Mean total cholesterol, mmol/L	4.57 (1.09)	4.58 (1.09)	4.58 (1.11)	0.937
Mean body mass index, kg/m^2	32.0 (6.50)	31.8 (6.45)	33.6 (6.70)	<0.001*
Macrovascular disease (%)				
None	1584 (90.8)	1419 (91.5)	165 (84.6)	0.002*
More than 1	161 (9.2)	131 (8.5)	30 (15.4)	
Smoking Status (%)				
Smoker	353 (21)	292 (19.6)	61 (32.4)	<0.001*
Non-Smoker	1324 (79)	1197 (80.4)	127 (67.6)	
Inflammatory markers				
Median CRP, mg/L	2.90 (1.20-6.40)	2.70 (1.10-6.18)	3.40 (1.55-9.35)	<0.001†
Median IL-4, ng/L	1.34 (1.11-1.68)	1.34 (1.10-1.68)	1.37 (1.21-1.70)	0.169
Median IL-6, ng/L	1.35 (0.68-3.42)	1.32 (0.67-3.33)	1.48 (0.80-5.92)	0.133
Median IL-10, ng/L	0.45 (0.34-0.63)	0.44 (0.34-0.63)	0.47 (0.34-0.68)	0.374
Median VEGF ,ng/L	75.3 (44.8-119.3)	74.73 (44.8-118.4)	82.22 (45.4-129.6)	0.588
Median TNF-α, ng/L	0.89 (0.39-1.86)	0.89 (0.39-1.87)	0.89 (0.43-1.79)	0.761
Median IL-1β, ng/L	1.02 (0.73-1.87)	1.01 (0.73-1.84)	1.15 (0.77-2.55)	0.123
Median IL-1RA, ng/L	437 (292.0-694.8)	429.1 (286.9-664.0)	529.3 (366.2-808.0)	<0.001†
Median MCP-1, ng/L	103.3 (60.2-152.9)	101.1 (59.2-151.1)	118.4 (66.3-166.6)	0.052
Median WBC, $x10^9/L$	6.51 (5.31-7.97)	6.45 (5.26-7.88)	6.91 (5.82-8.53)	<0.001†
Median adiponectin, mg/L	4.94 (3.28-7.50)	4.99 (3.27-7.62)	4.71 (3.30-6.49)	0.134

***significant α=0.05. †significant after Simes' improved Bonferroni correction for multiple testing. Missing or incomplete values for: HbA_{1c} = 117 cases, lipids = 245 cases, BMI = 3 cases, macrovascular history = 24 cases, smoking status = 92 cases, CRP = 308 cases, WBC = 183 cases, adiponectin = 304 cases. Values for IL-4, IL-6, IL-10, VEGF, TNF-α, IL-1β, IL-1RA, MCP-1 were for a subset of first 1227 subjects for whom there was a stored serum sample.**

Table 3.4: Unadjusted Spearman's ranked correlations for association between inflammatory marker concentration and PHQ-9 score for the baseline SOULD cohort (n=1227).

Inflammatory marker	r_s	p-value
CRP	0.15	<0.001*
IL-4	0.06	0.056
IL-6	0.05	0.075
IL-10	0.05	0.105
VEGF	0.07	0.012*
TNF-α	0.02	0.404
IL-1β	0.09	0.003*
IL-1RA	0.16	<0.001*
MCP-1	0.08	0.005*
WBC	0.13	<0.001*
TGL	0.11	<0.001*
Adiponectin	-0.07	0.010*

***significant after Simes' improved Bonferroni correction for multiple testing**

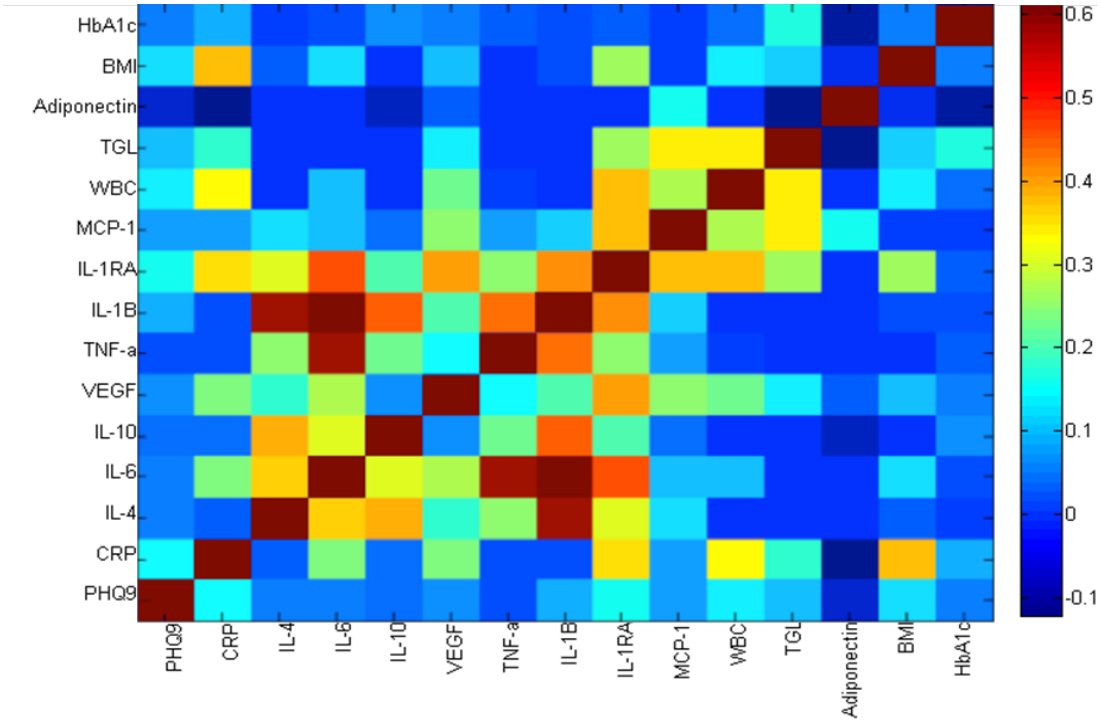


Figure 3.1 - Correlation Matrix showing unadjusted correlations of PHQ-9 score, inflammatory markers, BMI and HbA1c from table 3.5.

Table 3.5: Unadjusted Spearman's ranked correlations of inflammatory markers with each other, PHQ-9 score, BMI and HbA1c n = 1227

	PHQ-9	CRP	IL-4	IL-6	IL-10	VEGF	TNF-α	IL-1β	IL-1RA	MCP-1	WBC	TGL	Adiponectin	BMI	HbA1c
PHQ-9		.153	.055	.051	.046	.072	.024	.085	.158	.080	.134	.106	-.066	.124	.056
CRP	.153		.032	.235	.043	.236	.026	.022	.349	.076	.333	.181	-.113	.371	.092
IL-4	.055	.032		.365	.393	.179	.250	.580	.313	.124	.003	-.006	-.038	.029	.010
IL-6	.051	.235	.365		.306	.276	.581	.611	.460	.107	.103	-.025	-.034	.123	.020
IL-10	.046	.043	.393	.306		.070	.232	.448	.201	.045	-.002	-.019	-.076	-.016	.062
VEGF	.072	.236	.179	.276	.070		.146	.202	.401	.247	.232	.133	.031	.100	.058
TNF-α	.024	.026	.250	.581	.232	.146		.438	.250	.079	.014	.003	-.006	.002	.036
IL-1β	.085	.022	.580	.611	.448	.202	.438		.411	.112	-.013	-.003	-.035	.027	.022
IL-1RA	.158	.349	.313	.460	.201	.401	.250	.411		.377	.378	.256	-.005	.264	.037
MCP-1	.080	.076	.124	.107	.045	.247	.079	.112	.377		.276	.339	.164	.010	.005
WBC	.134	.333	.003	.103	-.002	.232	.014	-.013	.378	.276		.346	-.032	.141	.039
TGL	.106	.181	-.006	-.025	-.019	.133	.003	-.003	.256	.339	.346		-.121	.117	.171
Adiponectin	-.066	-.113	-.038	-.034	-.076	.031	-.006	-.035	-.005	.164	-.032	-.121		-.045	-.106
BMI	.124	.371	.029	.123	-.016	.100	.002	.027	.264	.010	.141	.117	-.045		.054
HbA1c	.056	.092	.010	.020	.062	.058	.036	.022	.037	.005	.039	.171	-.106	.054	

Correlation coefficients range from .000 (no correlation) to 1.00 (perfect correlation)

Table 3.5 and Figure 3.1 both report the correlations of the 12 inflammatory markers with BMI, HbA1c and PHQ-9 score. There is clustering of the associations of inflammatory markers with one another. For example, IL-10, IL-4 and IL-6, TNF-α and IL-1β are more closely correlated with one another than most other markers.

Table 3.6 reports the results of the final models of multiple linear regression analyses, which were used to compare concentrations of circulating markers of inflammation with levels of depressive symptoms (PHQ-9 score) while adjusting for socio-demographic factors, HbA_{1c}, adiposity (BMI), smoking, history of macrovascular disease and prescription of medication. Only those inflammatory markers that were associated with PHQ-9 score in unadjusted Spearman's Rho tests were included in the multiple linear regression analyses, namely, CRP, VEGF, IL-1β, IL-1RA, MCP-1, WBC, TGL and adiponectin. After adjusting for all covariates there remained a significant association between five inflammatory markers (CRP, IL-1RA, MCP-1, WBC, TGL) and depressive symptom score.

Table 3.6: Adjusted[†] final multiple linear regression models for the independent association between PHQ-9 score[‡] and each inflammatory marker[‡]

Model	R^2	Inflammatory marker		Age	Gender	BMI	MVD	Smoking
CRP	0.09	B (SE)	0.12 (0.04)	-0.01 (0.003)	0.19 (0.05)	0.006 (0.004)	0.25 (0.09)	0.31 (0.07)
		standardised-b	0.10	-0.16	0.10	0.04	0.07	0.13
		P	<0.001*	<0.001*	<0.001*	0.192	0.008*	<0.001*
VEGF	0.07	B (SE)	0.06 (0.04)	-0.01 (0.003)	0.14 (0.06)	0.008 (0.005)	0.19 (0.10)	0.32 (0.07)
		standardised-b	0.04	-0.15	0.07	0.05	0.06	0.13
		P	0.170	<0.001*	0.015*	0.072	0.048	<0.001*
IL-1β	0.07	B (SE)	0.07 (0.04)	-0.01 (0.003)	0.15 (0.06)	0.009 (0.005)	0.20 (0.10)	0.32 (0.07)
		standardised-b	0.05	-0.15	0.08	0.06	0.06	0.13
		P	0.081	<0.001*	0.011*	0.052	0.043	<0.001*
IL-1RA	0.08	B (SE)	0.17 (0.05)	-0.01 (0.003)	0.12 (0.06)	0.005 (0.005)	0.17 (0.10)	0.29 (0.07)
		standardised-b	0.12	-0.15	0.06	0.03	0.05	0.12
		P	<0.001*	<0.001*	0.051	0.310	0.082	<0.001*
MCP-1	0.07	B (SE)	0.15 (0.05)	-0.01 (0.003)	0.16 (0.06)	0.009 (0.005)	0.19 (0.10)	0.31 (0.07)
		standardised-b	0.10	-0.16	0.08	0.06	0.06	0.13
		P	0.002*	<0.001*	0.005*	0.055	0.052	<0.001*
WBC	0.09	B (SE)	0.32 (0.11)	-0.02 (0.003)	0.23 (0.05)	0.010 (0.004)	0.22 (0.09)	0.32 (0.07)
		standardised-b	0.08	-0.17	0.12	0.06	0.07	0.13
		P	0.003*	<0.001*	<0.001*	0.017	0.014*	<0.001*
TGL	0.09	B (SE)	0.22 (0.08)	-0.01 (0.002)	0.24 (0.05)	0.011 (0.004)	0.22 (0.09)	0.33 (0.06)
		standardised-b	0.07	-0.16	0.12	0.07	0.07	0.14
		P	0.007*	<0.001*	<0.001*	0.005*	0.011*	<0.001*
Adiponectin	0.08	B (SE)	-0.06 (0.05)	-0.01 (0.003)	0.24 (0.06)	0.009 (0.004)	0.26 (0.09)	0.34 (0.07)
		standardised-b	-0.04	-0.15	0.12	0.06	0.08	0.14
		P	0.210	<0.001*	<0.001*	0.023	0.004*	<0.001*

PHQ-9 score was entered as the dependent variable. Values of 'B', 'standardised-b' and 'p' presented here relate to the regression of column heading and PHQ-9 score. *significant after Simes' improved Bonferroni correction for multiple testing. †Adjusted for age, gender, ethnicity, HbA_{1c}, BMI, smoking, history of macrovascular disease (MVD) and prescribed medications. Only age, gender, BMI and MVD and Smoking are displayed here as these were the explanatory covariates. ‡These variables were ln transformed

Discussion

In a large population-based, newly diagnosed type 2 diabetes mellitus cohort, depression cases (as defined by the PHQ-9) were younger, more overweight, had a higher prevalence of macrovascular disease and had higher circulating concentrations of several established inflammatory markers. After adjusting for relevant potential confounding variables, including adiposity, age, gender, ethnicity, smoking, HbA_{1c}, diabetes complications and medications with anti-inflammatory action, the association between depressive symptoms and CRP, IL-1RA, MCP-1, WBC and TGL remained significant.

A strength of this study is that a large range of inflammatory markers were chosen *a priori* that have been implicated in the pathogenesis of type 2 diabetes mellitus and/or depression or depressive symptoms (Pickup et al., 1997; Ridker et al., 2002; Pickup and Mattock, 2003; Pickup, 2004; Kadowaki et al., 2006; Leo et al., 2006; Simon et al., 2008; Dowlati et al., 2010; Fernandez-Real and Pickup, 2012; Duivis et al., 2013; Lamers et al., 2013), the largest array of markers measured in any cohort of depression and type 2 diabetes mellitus. This allows for future analyses of the clustering of these markers and makes it possible to propose possible pathways that may be involved in the pathogenesis of depression and type 2 diabetes mellitus (if several markers from a similar pathway are associated with depressive symptom score). Biomarkers that are used in routine clinical practice were also chosen, including circulating CRP, WBC and TGL, which are all components of the acute-phase response in animals and humans and, as they are relatively easy to measure, may have clinical applications as useful biomarkers for depressive symptoms and acute/chronic stress (Steel and Whitehead, 1994; Pickup, 2004; Duivis et al., 2013). A population-based primary care sample was used, which aims to reduce selection bias. Using a cohort design allowed for the inclusion and adjustment for a range of potential confounding variables linking innate immunity, inflammation and depressive symptoms in type 2 diabetes mellitus, for example, diabetes-related

medications that have anti-inflammatory properties (statins, fibrates, systemic steroids and non-steroidal anti-inflammatory drugs). As these type 2 diabetes mellitus patients were newly diagnosed (less than 6 months) only 63 (4%) were on insulin therapy. Insulin therapy was equally prescribed in those who were depression cases versus those who were not but could otherwise have been a significant confounder – as insulin treatment is a known risk factor for depression and has anti-inflammatory effects. The cohort is representative of the multi-ethnic and socially diverse global type 2 diabetes mellitus population, and the setting was an inner city where the prevalence of both depression and type 2 diabetes mellitus are at their highest (Ayuso-Mateos et al., 2001). A continuous PHQ-9 score for depression was used to analyse the primary hypothesis both to improve the power of the analyses, and to overcome the limitations in the validity of using a threshold score to define depression. Two cut-offs were also used to determine cases of probable depression using the PHQ-9 that have general and population-specific validity for identifying cases of depression; that the results were similar when using these respective cut-offs demonstrates the robustness of the results.

The main limitation of the data is that they are cross-sectional, so a causative link between inflammatory markers, depressive symptoms and type 2 diabetes mellitus cannot be inferred. Additionally, up to a third of the sample population were excluded from the analyses because of missing data. Subjects that had missing data were significantly younger, and either had a higher HbA_{1c} or PHQ-9 for the CRP subset and other inflammatory marker subset respectively (Table 3.1 & Table 3.2). This may have led to an underestimation of the real differences in inflammation between depression cases and non-depressed subjects in the pair-wise comparisons. This may partly explain why, though there were associations between several inflammatory markers and PHQ-9 score, the only significant difference between depression cases and non-depressed subjects for the inflammatory marker subset was for IL-1RA. When defining a case of depression using a PHQ-9 cut-off ≥ 10 a diagnosis of major depression was not confirmed using a clinical interview. Thus, the prevalence of depression may have been may

have been overestimated because, although this cut-off has a high sensitivity, this comes at the expense of a lower specificity in diabetes patients (Twist et al., 2013). When using the cut-off ≥ 12, which has a better specificity for depression in this newly diagnosed type 2 diabetes mellitus population (Twist et al., 2013), to define cases of depression the prevalence of depression is lower (11.2%) and this may more closely reflect the prevalence of depression. As a continuous PHQ-9 score was used for the regressions, it unclear whether the reported associations between depressive symptom score and inflammation exist in both sub-threshold and clinically diagnosed depression. The prescription of antidepressants was not adjusted for because there is no systematic evidence from randomised controlled trials or observation studies to support a direct pro-inflammatory or anti-inflammatory effect. There is a further risk of residual confounding for poor adherence to medication, which it was not possible to measure, although medication data was derived from current GP prescription records. There was a small risk that comorbid acute or chronic inflammatory conditions may have been included, which could have led to an overestimation of effects, but terminal and advanced conditions were exclusion criteria so the more severely affected cases were excluded. The prescription of oestrogen replacement therapy or any potential inflammatory effects of pre-menopause were not adjusted for, but the majority of females in the study population were post-menopausal. It was not possible to include diet and physical activity because self-report measures are not sufficiently accurate but BMI was adjusted for, which may be considered a proxy marker for both.

In the general population, inflammation is significantly associated with cardiovascular disease (Ridker et al., 2002) and, since inflammation is also a significant biomarker associated with cardiovascular mortality in type 2 diabetes mellitus (Pickup and Mattock, 2003). It has been hypothesised that activated innate immunity is the common antecedent of a number of chronic non-communicable diseases such as type 2 diabetes mellitus, atherosclerosis and depressive symptoms (Pickup and Mattock, 2003), which tend to cluster together. It has been suggested that the increased risk of complications in type 2

diabetes mellitus with depression and depressive symptoms may be related to poorer blood glucose control (elevated HbA_{1c}) but in the present study concentrations of HbA_{1c} were not significantly raised in those who were depression cases. This group did appear to have developed diabetes at a younger age, which may or may not be due to behavioural effects of depression earlier in the lifespan. However, as this study is of newly diagnosed type 2 diabetes mellitus most patients have not had the opportunity to improve their glycaemic control, therefore the negative impact of the behavioural effects of depression on self-care (and glycaemic control) that others have observed may not be identifiable at this stage (Ciechanowski et al., 2000). It should be noted that although some collaborative care intervention studies (where therapies for both type 2 diabetes mellitus and depression are optimised) have shown improvements in HbA_{1c} (Katon et al., 2010), as a general rule glycaemic control does not improve when depression alone is treated (Ismail et al., 2007), which argues against the association between depression or depressive symptoms and poor glycaemic control being mediated by reduced self-care behaviours alone.

Inflammation may have a role in the pathogenesis of depression and depressive symptoms via numerous mechanisms, including altering the metabolism and activity of monoamine transmitters, effects on neurogenesis and neuroplasticity and activation of the hypothalamic-pituitary axis (Renn et al., 2011; Lamers et al., 2013). The increased concentrations of the inflammatory markers, associated in these newly diagnosed type 2 diabetes mellitus participants with increased depressive symptom scores (independent of HbA_{1c} and BMI), supports the alternate hypothesis that activated innate immunity is involved in the aetiology and pathogenesis of depression and/or the increased complications associated with it in type 2 diabetes mellitus.

IL-1β, a pro-inflammatory cytokine, was positively associated with depressive symptoms in the unadjusted analyses (the magnitude of the PHQ-9 score), as was IL-1RA, which blocks IL-1β from binding to its receptor. Where there is increased

inflammation and IL-1β activity in specific tissues this leads to increased IL-1RA secretion (Arend, 2002). That both these inflammatory markers were similarly associated with depressive symptom score is consistent with the described pathway.

Adiponectin, negatively associated with depressive symptom score in this chapter, is known to have an insulin sensitizing effect, to have anti-inflammatory effects, and has been reported as decreased in depression (Kadowaki et al., 2006; Leo et al., 2006; Villarreal-Molina and Antuna-Puente, 2012). In this chapter the association between adiponectin and depressive symptom score was attenuated when adjusting for BMI - BMI was also a significant explanatory covariate in the final models for the association of PHQ-9 with WBC and TGL. This suggests that some of the increased concentrations of inflammatory markers and the reduced concentrations of adiponectin that were detected in this group are associated with the increased adiposity.

Only the inflammatory markers WBC, CRP, IL-1RA, MCP-1 and TGL were significantly associated with depressive symptom score; whether these markers represent a cluster or subtype of the innate inflammatory response more likely to be associated with depressive symptoms needs to be investigated. No association between some markers (such as IL-6 and TNF-α) were detected, however IL-6 and TNF-α have been previously associated with both depression and depressive symptoms, and with type 2 diabetes mellitus (Zeugmann et al., 2010; Doyle et al., 2013). There are a number of possibilities that could explain this and there is a risk of over speculating. It is possible that that there are subtypes of depression characterised by different inflammatory profiles. It may be that the concentrations of cytokines such as IL-6 and TNF-α (which are raised in type 2 diabetes mellitus and have been associated with its onset) are already raised to a significant level in the study population and in combination with the other markers (which are associated with depressive symptom score in the cohort) characterise the increased depressive symptoms in the sample.

Additionally, the circadian rhythm of IL-6 has been shown to fluctuate sharply throughout the day in those with depression, most probably due to HPA axis hyperactivity (Alesci et al., 2005). As time of venepuncture was not controlled, temporal variation of IL-6 concentrations may explain why there was only a non-statistically significant 0.16 ng/L difference between those that were depression cases and those that were non-depressed subjects.

The between-group differences in inflammatory markers when using the PHQ-9 cut-off of ≥ 10 and 12 were almost identical. Although the lower cut-off risks inclusion of non-depressed subjects in the smaller depression group (discussed in Chapter 2), this has not masked any differences in inflammation between the two groups. The cut-off of ≥ 10 was used to determine depression cases for the remainder of the thesis as this is the most accepted cut-off and there were no clear benefits to using the cut-off ≥ 12.

In conclusion, these findings support the hypothesis that inflammation in type 2 diabetes mellitus may cause the parallel development of depressive symptoms, glucose intolerance and atherosclerosis (Ridker et al., 2002; Pickup and Mattock, 2003). People with more depressive symptoms and a recent diagnosis of type 2 diabetes mellitus have higher circulating concentrations of inflammatory markers than people with diabetes with less depressive symptoms.

The next chapter will examine whether groups of inflammatory markers are more closely associated with depression symptom score than any individual marker. Future chapters will examine whether the concentrations of inflammatory markers at diagnosis of diabetes predict the later development of depressive symptoms and also whether persistent depression is associated with increased concentrations of inflammatory markers 12 months after diagnosis with type 2 diabetes mellitus.

Chapter 4: Exploratory Factor Analysis and Composite Scores of Inflammatory Markers

Synopsis

This aim of this chapter is to examine the factor structure of depressive symptom score and an array of inflammatory markers at baseline in a newly diagnosed type 2 diabetes mellitus population. The hypothesis that some inflammatory markers will cluster together and that composite z-scores of these individual markers are better correlated with depressive symptom score than any individual inflammatory marker was tested. Exploratory maximum likelihood factor analysis was used to determine the factor structure of depressive symptoms and inflammatory markers and the association between each inflammatory marker and depressive symptom score was estimated by Pearson's correlations and multiple linear regressions. Covariates adjusted for in the multiple linear regressions included, socio-demographic factors, adiposity, macrovascular disease, HbA_{1c} and prescribed medication. Five factors were identified in the newly diagnosed type 2 diabetes mellitus sample population. The five factors were labelled, 'PHQ-9 1' (for depressive symptoms), 'PHQ-9 2' (for depressive and diabetes symptoms), 'cytokine 1', 'cytokine 2'and 'cytokine 3' respectively. Composite z-scores of the 'cytokine 2' factor were significantly associated with PHQ-9 score and accounted for more of the variance in PHQ-9 score than individual inflammatory marker. These findings suggest that this composite measure of inflammation may be more powerful at predicting future depression in this type 2 diabetes mellitus population.

Introduction

Depression is twice as common in type 2 diabetes mellitus compared to those in the general population (Anderson et al., 2001; Nichols and Brown, 2003) and the association between depression and type 2 diabetes mellitus is bi-directional, with as much as a 60% increased incidence of type 2 diabetes mellitus in those with depression and a 15% risk of incident depression in those with type 2 diabetes mellitus (Leo et al., 2006; Golden et al., 2008; Mezuk et al., 2008). Epidemiological studies have shown that those with type 2 diabetes mellitus and depression have worse glycaemic control (Lustman et al., 2000) and an increased incidence of diabetes complications and mortality (Katon et al., 2005; Winkley et al., 2012). The poor prognosis of those with depression and type 2 diabetes mellitus highlights the need to better understand which biological processes are underlying this comorbidity.

There are several potential mechanisms that could explain aspects of the bi-directional relationship between depression and type 2 diabetes mellitus (Ismail, 2010; Renn et al., 2011). One potential biological mechanism could be the systemic cytokine-mediated chronic inflammatory state, which is independently associated with both the onset of type 2 diabetes mellitus (Pradhan et al., 2001; Freeman et al., 2002; Thorand et al., 2003; Pickup, 2004), progression of type 2 diabetes mellitus (Pickup et al., 1997; Fernandez-Real and Pickup, 2012), established depression (Simon et al., 2008; Howren et al., 2009; Miller et al., 2009; Dowlati et al., 2010; Lamers et al., 2013) and comorbid depression in both newly diagnosed and established type 2 diabetes mellitus (Laake et al., 2014).

In the previous chapter, five of the 12 inflammatory markers measured were independently associated with depression in newly diagnosed type 2 diabetes mellitus. It is not clear whether concentrations of some of these markers are closely related to each other and depressive symptom score, or why some markers are not associated with depressive symptom score. It is possible that the

variability of the concentrations of several of these inflammatory markers together may be represented by a smaller group of underlying or latent variables.

One method for identifying whether a large number of observed variables have an underlying grouping is latent (hidden) variable analysis. The most appropriate method for continuous data where there is no *a priori* hypothesis about underlying factor patterns is exploratory factor analysis. This method is based on the common factor model and searches for joint variation in response to unobserved latent variables (Costello and Osborne, 2005). It can identify the latent construct variables (factors) that underlie a set of measured variables, using a maximum likelihood method with an oblique rotation accounts for correlation between factors and the individual variables and measures the inter-factor correlations (Costello and Osborne, 2005). Determining the factor structure of the inflammatory markers, and the depressive symptoms measured by the PHQ-9, will further enhance the understanding of any underlying pattern of inflammatory markers and their association with depression in type 2 diabetes mellitus and could also lead to the generation of new hypotheses.

Latent constructs can be used to create aggregate scores of inflammation. A statistically valid method of doing this is to add the z-scores for each variable to be included in the aggregate score - in this case each inflammatory marker concentration (Streiner and Norman, 2008). Although aggregate scores to measure depressive symptoms and many other psychological or physical symptoms are commonly used, such as the PHQ-9 for depression (Spitzer et al., 1999) and the HADS for anxiety and depression (Zigmond and Snaith, 1983), the majority of studies of the association between biomarkers and depression compare concentrations of individual markers with depressive symptom score (Lamers et al., 2013; Laake et al., 2014).

Methods

Design, Setting, Sampling frame: The study design, setting and sampling frame were the same as for the previous chapter (Chapter 3) and have been previously described in the methods chapter (Chapter 2).

Study population and case definition: The study population and case definition were the same as for the previous chapter (Chapter 3). Recruitment and baseline appointments for data presented in this chapter were conducted between May 2008 and September 2012; further details of the sampling methodology have been described in Chapter 2.

Variables: Eleven markers of inflammation which had been identified *a priori* as associated with type 2 diabetes mellitus and/or depression (CRP, IL-4, IL-6, IL-10, VEGF, TNF-α, IL-1β, IL-1RA, MCP-1, WBC and TGL) were included in the analysis, adiponectin was excluded because it has a negative association with depressive symptom score and so could not be included in an index. Depression was measured using the PHQ-9, a self-report measure that was developed for primary care to aid clinicians in identifying probable cases of depression. PHQ-9 scores ≥ 5, 10, 15, and 20 represented mild, moderate, moderately severe, and severe depression respectively (Kroenke et al., 2001), and the PHQ-9 has demonstrated validity in type 2 diabetes mellitus populations (Twist et al., 2013). Detailed methods and a description of each of these variables are provided in full in the methods chapter (Chapter 2).

Assessment of confounders: Confounders included were the same as those in Chapter 3: age, gender, history of macrovascular disease (MI, CABG, CVA, carotid or limb re-vascularisation), current prescribed medications with a possible anti-inflammatory action (statins, fibrates, systemic steroids, non-steroidal anti-inflammatory drugs, COX-2 inhibitors), HbA_{1c}, body mass index (kg/m^2),

smoking status, self-report ethnicity. Detailed methods for collection of these variables and of their relevance as confounders have been previously described in Chapter 2 and Chapter 3.

Statistical Analyses: Data were analysed using SPSS 21.0 (IBM Corp. Released 2011. IBM SPSS Statistics, Version 21.0. Armonk, NY). Main characteristics of the population are summarised as mean (SD) where data were normally distributed or median (IQR) where data were skewed, or as a count (percentage) for categorical variables, all stratified by depression status. Statistical analyses were conducted using Student's *t* test for normally distributed continuous data, Mann-Whitney U analyses for non-normally distributed continuous data and r_s to compare bivariate continuous variables. χ^2 test was used for comparisons of categorical data. A natural log was used to transform skewed data in multiple regressions and to transform inflammatory markers for the factor analysis.

Exploratory maximum likelihood factor analysis was used to derive the factor structure of the PHQ-9 depressive symptom score and inflammatory markers. Initially, a correlation matrix of the nine symptoms that make up the PHQ-9 depressive symptom score and the 11 inflammatory markers was generated. Participants with missing values for any of the PHQ-9 score items or any of the 11 inflammatory markers were excluded, as they could not contribute to all of the correlation coefficients within the matrix.

Secondly, the factors were extracted. An exploratory maximum likelihood method was chosen as a suggested factor structure of PHQ-9 items and inflammatory markers had not been previously determined. In this step factors were extracted sequentially according to the proportion of the variance they account for. Each additional factor was subsequently extracted from the residual matrix after the variance from the previous factors has been removed. The final step was to apply a rotation to allow for a simple factor structure; an oblique

rotation was chosen. Oblique rotation was used as it assumes the factors are correlated with one another, as opposed to an orthogonal rotation (which assumes factors are not correlated). A value of 0.3 was used as the cut-off when identifying factor loadings, although 0.4 is the typical cut-off to identify factor loadings 0.3 is justified if the sample size is > 350 (Hair, 2006).

The purpose of this factor analysis was to identify whether inflammatory markers grouped together in order to create composite scores of inflammation, therefore it was necessary to limit the number of factors which are extracted in the rotation. A five-factor design was decided upon based on a Cattel's Scree Test, as using Kaiser criterion (only including factors with eigenvalues >1) could have excluded factors that still accounted for a significant proportion (5%) of the variance. Finally, each factor was labelled according to the variables it contained.

From the results of the factor analysis three index scores of inflammatory markers were created. Index scores were created by transforming each inflammatory marker concentration into a z-score, before combining the z-scores for every marker included in the index (Streiner and Norman, 2008). Scores were not weighted as it has been widely reported that weighting scores in health measurement scales has little or no benefit (Streiner and Norman, 2008). Multiple linear regression was used to assess the relationship between depressive score as the dependent variable and inflammatory marker index; covariates were added to the model in sequential steps using a hierarchical method. Simes' improved Bonferroni method was used to correct for multiple testing for pair-wise comparisons of inflammatory marker differences between groups and association between inflammatory marker concentration and PHQ-9 score. An assessment of the residuals did not suggest major violations of the assumptions of a multiple regression.

Results

The sample population and baseline characteristics of the participants have been described previously in Chapter 2 and Chapter 3. Analyses in this chapter were carried out on a subset of participants n = 1083 for whom there was a complete set of inflammatory marker assays and PHQ-9 score (60% of the total) as they could not contribute to the factor analysis (see methods) and so that fair comparisons could be made on the amount of variance explained by each marker and the composite scores. Compared to those with complete blood results, those who had missing or un-analysable blood samples or incomplete PHQ-9 scores (n = 686) were younger and more likely to be of Black African or Caribbean ethnicity and had a higher PHQ-9 score. There were no statistically significant differences in gender, PHQ-9 derived depression status or HbA_{1c} (table 4.1).

Table 4.1: Baseline characteristics of participants with type 2 diabetes mellitus in the South London Diabetes Study with a PHQ-9 score at baseline stratified by whether subjects also had a complete set of inflammatory markers assayed

Baseline variable	Total (n = 1769)	All Tests Complete (n = 1083)	Missing Tests (n = 686)	p-value
Mean age, years	56.1 (11.04)	57 (11.04)	55 (11.04)	<0.001*
Gender (%)				
Male	976 (55.2)	606 (56.0)	370 (53.9)	0.405
Female	793 (44.8)	477 (44.0)	316 (46.1)	
Ethnicity (%)				
White	878 (49.6)	587 (54.2)	291 (42.4)	<0.001*
Black	710 (40.1)	385 (35.5)	325 (47.4)	
Asian/Other	181 (10.2)	111 (10.2)	70 (10.2)	
HbA_{1c}				
Mean % HbA_{1c}	7.00 (1.45)	6.97 (1.40)	7.07 (1.54)	0.156
Mean HbA_{1c}, mmol/mol	53.1 (15.9)	52.7 (15.3)	53.8 (16.8)	
Psychological assessment				
PHQ-9 score	3.0 (0.0-6.0)	2.0 (0.0-6.0)	3.0 (0.0-6.0)	0.029*
PHQ-9 depression (%)	258 (14.6)	150 (13.9)	108 (15.7)	0.272

***significant α=0.05. T-test and Mann Witney U test used for normal and non-normal data**

Table 4.2: Characteristics of 1083 depressed (PHQ-9≥ 10) and non-depressed (PHQ-9 < 10) subjects with type 2 diabetes mellitus in the South London Diabetes Cohort Study for whom a complete set of inflammatory markers were assayed

Baseline variable	Total (n = 1083)	No depression (n = 933)	Depression (n = 150)	p-value
Mean age, years	56.8 (10.99)	57.3 (11.09)	53.9 (9.90)	<0.001*
Gender (%)				
Male	606 (56.0)	527 (56.5)	79 (52.7)	0.382
Female	477 (44.0)	406 (43.5)	71 (47.3)	
Ethnicity (%)				
White	587 (54.2)	506 (54.2)	81 (54.0)	0.222
Black	385 (35.5)	337 (36.1)	48 (32.0)	
Asian/Other	111 (10.2)	90 (9.6)	21 (14.0)	
[1]Mean glycated haemoglobin, mmol/mol	52.7 (15.33)	52.4 (15.26)	54.3 (15.70)	0.147
[2]Lipids				
Median triglyceride, mmol/L	1.40 (1.00-2.00)	1.40 (0.90-1.90)	1.50 (1.10-2.40)	0.009*
Mean low density lipoprotein, mmol/L	2.66 (0.90)	2.66 (0.88)	2.64 (0.98)	0.830
Mean high density lipoprotein, mmol/L	1.20 (0.34)	1.21 (0.33)	1.20 (0.41)	0.770
Mean total cholesterol, mmol/L	4.61 (1.08)	4.59 (1.04)	4.71 (1.23)	0.285
[3]Mean body mass index, kg/m²	31.8 (6.41)	31.6 (6.21)	32.9 (7.46)	0.041*
[4]Macrovascular disease (%)				
None	968(90.6)	843 (91.6)	125 (84.5)	0.005*
More than 1	100 (9.4)	77 (8.4)	23 (15.5)	

Data are n (%), mean (SD) or median (IQR) as appropriate
***significant α=0.05 . T-test and Mann Witney U test used for normal and non-normal data**
[1]missing data for this variable, HbA_{1c} values were not available for 12 cases.
[2]missing data for this variable, LDL vales were not available for 89 cases, HDL values were not available for 57 cases and total cholesterol values were not available for 30 cases.
[3]missing data for this variable, BMI values were not available for 1 case.
[4]missing data for this variable: data on macrovascular history was unavailable for 15 cases.

Table 4.2 reports the demographic characteristics of this group of 1083 type 2 diabetes mellitus patients with and without depression. The differences in demographics between those with depression and those without were similar to that of the 1769 participants, previously described in Chapter 3 (Laake et al., 2014).

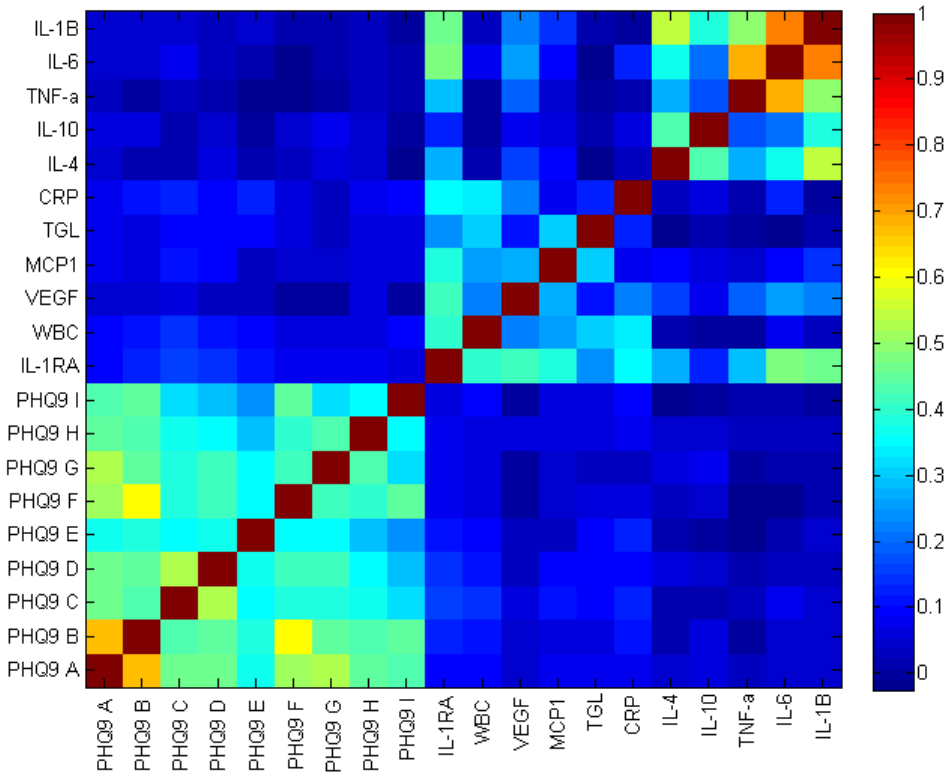


Figure 4.1 - Correlation matrix for PHQ-9 score and inflammatory markers

Figure 4.1 and Table 4.3 are correlation matrices for PHQ-9 score and inflammatory markers. It shows three clusters of cytokines and two clusters of PHQ-9 score questions. The PHQ-9 score items were all very closely correlated with each other, and less so with the inflammatory markers, which are closely correlated with each other in two to three visible clusters.

Table 4.3: Spearman's Correlations of Inflammatory Markers and individual PHQ-9 symptoms with each other (n = 1083)

	PHQA	PHQB	PHQC	PHQD	PHQE	PHQF	PHQG	PHQH	PHQI	IL-1RA	WBC	VEGF	MCP-1	TGL	CRP	IL-4	IL-10	TNF-α	IL-6	IL-1β
PHQA		.666	.465	.462	.372	.509	.523	.450	.437	.100	.093	.043	.073	.078	.073	.039	.065	.028	.042	.055
PHQB	.666		.438	.454	.384	.614	.449	.436	.452	.122	.118	.047	.060	.057	.110	.021	.070	.005	.045	.043
PHQC	.465	.438		.528	.352	.389	.388	.362	.314	.156	.149	.061	.116	.096	.134	.021	.020	.032	.084	.054
PHQD	.462	.454	.528		.369	.421	.421	.359	.292	.146	.118	.036	.102	.093	.101	.059	.048	.017	.027	.025
PHQE	.372	.384	.352	.369		.350	.356	.288	.238	.109	.092	.024	.023	.095	.132	.015	.005	-.025	.007	.039
PHQF	.509	.614	.389	.421	.350		.420	.402	.453	.075	.063	-.003	.040	.065	.066	.034	.051	-.024	-.011	.008
PHQG	.523	.449	.388	.421	.356	.420		.437	.322	.080	.066	-.002	.043	.037	.032	.069	.073	.004	.010	.013
PHQH	.450	.436	.362	.359	.288	.402	.437		.353	.079	.056	.065	.064	.065	.073	.042	.051	.025	.036	.025
PHQI	.437	.452	.314	.292	.238	.453	.322	.353		.068	.098	-.009	.056	.068	.100	-.010	.000	.009	.009	.003
IL-1RA	.100	.122	.156	.146	.109	.075	.080	.079	.068		.391	.418	.388	.242	.348	.265	.134	.294	.476	.456
WBC	.093	.118	.149	.118	.092	.063	.066	.056	.098	.391		.225	.254	.300	.342	.015	-.008	.004	.081	.027
VEGF	.043	.047	.061	.036	.024	-.003	-.002	.065	-.009	.418	.225		.271	.116	.215	.164	.073	.193	.262	.228
MCP-1	.073	.060	.116	.102	.023	.040	.043	.064	.056	.388	.254	.271		.298	.075	.095	.068	.052	.100	.146
TGL	.078	.057	.096	.093	.095	.065	.037	.065	.068	.242	.300	.116	.298		.131	-.016	.020	.000	-.013	.012
CRP	.073	.110	.134	.101	.132	.066	.032	.073	.100	.348	.342	.215	.075	.131		.035	.055	.020	.120	-.009
IL-4	.039	.021	.021	.059	.015	.034	.069	.042	-.010	.265	.015	.164	.095	-.016	.035		.436	.271	.363	.545
IL-10	.065	.070	.020	.048	.005	.051	.073	.051	.000	.134	-.008	.073	.068	.020	.055	.436		.175	.208	.383
TNF-α	.028	.005	.032	.017	-.025	-.024	.004	.025	.009	.294	.004	.193	.052	.000	.020	.271	.175		.695	.496
IL-6	.042	.045	.084	.027	.007	-.011	.010	.036	.009	.476	.081	.262	.100	-.013	.120	.363	.208	.695		.740
IL-1β	.055	.043	.054	.025	.039	.008	.013	.025	.003	.456	.027	.228	.146	.012	-.009	.545	.383	.496	.740	

Correlation coefficients range from .000 (no correlation) to 1.00 (perfect correlation)

Table 4.4 shows the classification of cytokines and PHQ-9 symptoms after the exploratory factor analysis. The PHQ-9 score was segregated into two clusters. The first cluster included the following symptoms: depressed mood, feelings of worthlessness or guilt, anhedonia, suicidal ideation, thought of death, psychomotor agitation or retardation. The second cluster of PHQ-9 score symptoms included symptoms of fatigue or loss of energy, insomnia or hypersomnia, loss of appetite and diminished ability to concentrate. The factor analysis also revealed three clusters of inflammatory markers, within each of which inflammatory markers were well correlated with each other. The first cluster included IL-6, IL-1β and TNF-α, the second cluster included CRP, IL-1RA, WBC, MCP-1, VEGF and TGL and the third cluster included IL-4, IL-10 and IL-1β. Of these three clusters cluster 'cytokine 1' was slightly correlated with PHQ-9 score, cluster 'cytokine 2' was more strongly correlated with PHQ-9 score than any individual marker, and cluster 'cytokine 3' was slightly correlated with PHQ-9 score (Table 4.5).

Table 4.4: Pattern matrix of depressive symptoms and inflammatory markers after factor analysis

Depressive Symptoms & Inflammatory Markers	PHQ-9 Symptoms		Cytokine Classification		
	Factor PHQ-9 1	Factor PHQ-9 2	Factor Cytokine 1	Factor Cytokine 2	Factor Cytokine 3
Depressed mood	**0.90**	-0.07	0.01	0.03	0.01
Feelings of worthlessness or guilt	**0.68**	0.05	-0.05	-0.02	0.04
Anhedonia	**0.65**	0.17	0.01	-0.02	0.04
Suicidal ideation or thoughts of death	**0.57**	-0.01	0.01	0.03	-0.04
Psychomotor agitation or retardation	**0.35**	0.27	0.02	-0.02	0.02
Fatigue or loss of energy	-0.01	**0.73**	-0.01	0.02	0.02
Insomnia or hypersomnia	-0.01	**0.69**	0.08	0.05	-0.05
Diminished ability to concentrate	0.28	**0.40**	-0.02	-0.06	0.06
Loss of appetite	0.18	**0.36**	-0.02	0.04	0.14
IL-6	0.01	0.03	**0.99**	0.05	0.01
TNF-α	-0.03	0.04	**0.70**	-0.03	0.02
IL-1β	0.01	-0.22	**0.52**	0.06	**0.50**
IL-1RA	0.01	0.01	0.25	**0.68**	0.15
WBC	0.04	0.01	-0.02	**0.59**	-0.10
MCP-1	-0.04	0.04	-0.07	**0.49**	0.12
CRP	0.06	0.01	0.07	**0.44**	-0.13
VEGF	0.02	-0.07	0.13	**0.44**	0.08
TGL	-0.02	0.05	-0.11	**0.42**	-0.01
IL-10	0.06	-0.03	-0.03	0.03	**0.68**
IL-4	-0.05	0.05	0.08	-0.01	**0.57**

The factor order (percentage of variance explained) was 'cytokine 1' (23.0%), 'PHQ-9 1' (16.2%), 'cytokine 2' (9.5%), 'cytokine 3' (5.5%) and 'PHQ-9 2'(5.0%).

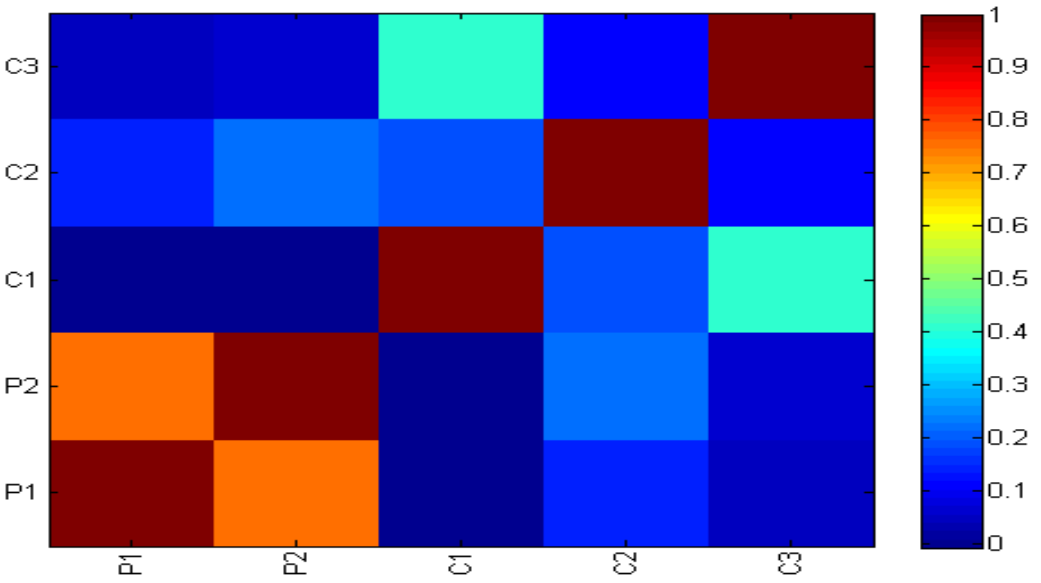


Figure 4.2 - Correlation matrix of the five extracted factors.

Table 4.5 Table showing correlations between the five extracted factors

	P1	P2	C1	C2	C3
P1	1.000	.762	.001	.139	.047
P2	.762	1.000	-.009	.218	.057
C1	.001	-.009	1.000	.192	.408
C2	.139	.218	.192	1.000	.107
C3	.047	.057	.408	.107	1.000

Correlation coefficients range from .000 (no correlation) to 1.00 (perfect correlation)

Figure 4.2 and Table 4.5 show the correlations between the five extracted factors. Factors 'PHQ-9 1' and 'PHQ-9 2' are very closely correlated (0.76), factor 'cytokine 2' is the most closely correlated with factors PHQ-9 1 and PHQ-9 2 (0.14-2.18).

Table 4.6: Unadjusted Spearman's ranked correlations for the independent association between inflammation indices or inflammatory marker concentration and PHQ-9 score

Composite inflammation indices	r_s	p-value
Cytokine Index 1 (IL-6, IL-1β, TNF-α)	0.09	0.005*
Cytokine Index 2 (CRP, IL-1RA, WBC, MCP-1, VEGF, TGL)	0.19	<0.001*
Cytokine Index 3 (IL-4, IL-10, IL-1β)	0.10	0.001*
Individual inflammatory marker		
CRP	0.12	<0.001*
IL-4	0.08	0.013*
IL-6	0.06	0.047
IL-10	0.07	0.029*
VEGF	0.08	0.013*
TNF-α	0.04	0.175
IL-1β	0.10	0.002*
IL-1RA	0.17	<0.001*
MCP-1	0.10	0.001*
WBC	0.14	<0.001*
TGL	0.09	0.002*

***significant after Simes' improved Bonferroni correction for multiple testing**

Table 4.6 reports bivariate correlations between PHQ-9 score and the inflammatory index score as well as the individual correlations with respective inflammatory markers. The inflammatory index score 'cytokine 2' (CRP, IL-1RA, WBC, MCP-1, VEGF and TGL) was correlated more strongly with PHQ-9 score than any of the markers individually. The inflammatory index score composed of IL-6, IL-1β, and TNF-α was weakly correlated with depressive symptom score and no more strongly correlated than the markers alone.

Table 4.7 reports the results of the final models of multiple linear regression analyses (MLR), these were used to compare the inflammatory indices and concentrations of individual circulating markers of inflammation with levels of depressive symptoms (PHQ-9 score) while adjusting for socio-demographic factors, HbA_{1c}, adiposity (BMI), smoking history, history of macrovascular disease and prescription of medication. After adjusting for all covariates there remained a significant association between seven inflammatory markers (CRP, IL-4, IL-10, IL-1RA, MCP-1, WBC, TGL) and depressive score. There was not a significant association between the inflammatory markers, IL-6, IL-1β, and TNF-α. The inflammatory index including CRP, IL-1RA, WBC, MCP-1, VEGF and TGL explained as much as twice as much of the variance of depressive symptom score as any of the other markers alone. The remaining two inflammatory indices did not explain any more of the variance of depressive symptom score than any of the markers alone.

Table 4.7: Adjusted[1] final multiple linear regression models for the independent association between PHQ-9 score[2] and each inflammatory marker[2] or inflammatory score

Model	R^2		
Cytokine Index 1 (IL-6, IL-1β, TNF-α)	0.07	B (SE) standardised-b p	0.02 (0.01) 0.04 0.145
Cytokine Index 2 (CRP, IL-1RA, WBC, MCP-1, VEGF, TGL)	0.09	B (SE) standardised-b p	0.04 (0.01) 0.17 <0.001*
Cytokine Index 3 (IL-4, IL-10, IL-1β)	0.07	B (SE) standardised-b p	0.03 (0.01) 0.07 0.028*
CRP	0.07	B (SE) standardised-b p	0.12 (0.04) 0.10 0.004*
IL-4	0.07	B (SE) standardised-b p	0.26 (0.12) 0.07 0.029*
IL-10	0.06	B (SE) standardised-b p	0.15 (0.10) 0.05 0.133
VEGF	0.07	B (SE) standardised-b p	0.06 (0.04) 0.04 0.173
IL-1β	0.07	B (SE) standardised-b p	0.07 (0.05) 0.05 0.115
IL-1RA	0.07	B (SE) standardised-b p	0.19 (0.05) 0.13 <0.001*
MCP-1	0.08	B (SE) standardised-b p	0.18 (0.05) 0.12 0.001*
WBC	0.08	B (SE) standardised-b p	0.40 (0.12) 0.11 0.001*
TGL	0.07	B (SE) standardised-b p	0.17 (0.10) 0.06 0.087

[1]adjusted for age, gender, ethnicity, HbA_{1c}, BMI, smoking, history of macrovascular disease (MVD) and prescribed medications. [2]These variables were ln transformed
***significant after Simes' improved Bonferroni correction for multiple testing**

Discussion

Increased concentrations of inflammatory markers are associated with depression in type 2 diabetes mellitus. In Chapter 3, five inflammatory markers were identified that were raised in depression in newly diagnosed type 2 diabetes mellitus. Here the structure of the relationships between 11 inflammatory markers and the nine depressive symptoms measured by the PHQ-9 were examined. Factor analysis identified three clusters of inflammatory markers from which three composite scores of inflammatory markers were created which were then compared with a PHQ-9 depressive symptom score in participants with newly diagnosed type 2 diabetes mellitus. As expected, depressive symptoms were more closely correlated with a score of these markers than each individually. In multiple regression analyses two of the three inflammation scores were independently associated with depressive symptom score and one explained substantially more of the variance of PHQ-9 score than any marker individually.

The main strength of this study is its large sample size (n = 1083). When using smaller sample sizes (less than 300 cases) for factor analysis the correlation coefficients between variables are generally less reliable (Tabachnick and Fidell, 2006). Additionally, the most conservative recommendations are to include at least 10-20 times as many subjects as the number of variables included in the factor analysis (*N:p* = 10-20) (MacCallum et al., 1999; Hair, 2006). Considering this analysis included 20 variables and 1083 subjects, a further strength is the large *N:p* of 54. Factor analysis is also limited by the quality of the data used, the measures used for depression have been validated for use in primary care and newly diagnosed diabetes populations (Spitzer et al., 1999; Twist et al., 2013) and the measures of inflammation are robust and have low inter-assay and intra-assay coefficients of variation. Strengths previously discussed in Chapter 3 which are also applicable to this study, as it is a subsection of the same dataset, include the following: a population-based primary care sample to reduce selection bias, a cohort that is representative of the multi-ethnic and social diversity of the global

type 2 diabetes mellitus epidemic, a large number of inflammatory markers associated *a priori* with depression and/or diabetes were included in this analysis, continuous measures.

The main limitation of this factor analysis is that the associations described cross-sectional relationships, therefore direction of any association between inflammatory markers or between inflammatory markers and PHQ-9 score symptoms cannot be assessed. In this factor analysis the correlations between the factors were mostly weak, however the large sample size gives confidence that the extracted factors are statistically valid (MacCallum et al., 1999). The factor structure was derived from 11 inflammatory markers and nine depressive symptoms; however, the association between several of these inflammatory markers (e.g. VEGF) and depressive symptom score disappeared after adjusting for confounders. After adjusting for confounders, Cytokine Index 2 may have explained even more of the variance of PHQ-9 score had it not included VEGF (as it would then have consisted only markers which were independently associated with depressive symptom score). Although there was a relatively large sample size for the factor analysis, unfortunately 40% of the sample population were excluded due to missing data. Subjects that had missing data were significantly younger and had a higher PHQ-9 score. Therefore, these results may be less applicable to the younger or more severely depressed type 2 diabetes mellitus population, and there may have been an underestimation of the size of the correlations. The limitations already discussed in Chapter 3 that are also applicable to this study are summarised below. Participants who were receiving antidepressant therapy at the time of interview may have been non-symptomatic for depression; this may mask the extent of an association between inflammation and depressive symptoms. Prescribed medications that may have an anti-inflammatory effect were adjusted for, but it was not possible to measure adherence to these drugs. Finally, as a continuous PHQ-9 score has been used, it unclear whether the reported associations between depressive symptom score and inflammation index exist in both sub-threshold and clinically diagnosed depression.

In type 2 diabetes mellitus a range of inflammatory markers have been associated with depression, of these CRP, IL-1RA, WBC, MCP-1, VEGF and TGL seem to have a similar effect so that, independently of significant covariates, including HbA_{1c} and BMI, having raised concentrations of several of these markers is more strongly associated with depressive symptom score than any individual inflammatory marker alone. This adds weight to the hypothesis that activated innate immunity is involved in the aetiology and pathogenesis of depression and/or the increased complications associated with depression in type 2 diabetes mellitus.

Of the markers that were included in this score, WBC and MCP-1 suggest that leukocytes are particularly important in the association between depression and type 2 diabetes mellitus. That CRP and IL-1RA are both grouped in the same factor together suggests that the described pathway involving CRP, IL-6, IL-1β and IL-1RA is also important in this association. As both IL-6 and IL-1β were measured it was surprising that they did not cluster with CRP and IL-1RA in the factor analysis. It may be that IL-6 and IL-1β are expressed more in specific tissues but that circulating concentrations are not as representative of the tissue specific expression and thus circulating concentrations are less useful. An additional possibility may be that the longer times in transport, which are unavoidable in primary care based clinical research, and more sample degradation may mean that measurements of these markers were less accurate than previous studies.

The PHQ-9 depressive symptom score was originally designed for use in the general population as a screening tool from depression, when used in this context the PHQ-9 has a factor structure of one (Huang et al., 2006). The PHQ-9 has been used commonly in epidemiological research as a self-report scale depressive symptom score. Recent research in other depression samples with chronic comorbid conditions has proposed a two-factor structure for the

PHQ-9. One factor consists of 'cognitive' symptoms more commonly associated with the construct of depression and the other factor consists of 'whole body' symptoms which are associated with the construct of depression and also commonly with associated with chronic illness (fatigue, sleep disturbance and loss of appetite) (Krause et al., 2008; Richardson and Richards, 2008). Interestingly the depressive symptoms which have been grouped together in factor 'PHQ-9 1' are those which are unlikely to be symptoms of anything other than depression, while cluster 'PHQ-9 2' consists of symptoms which could also be symptoms of type 2 diabetes mellitus or symptoms which are related to it (the three symptoms previously described and loss of concentration). This suggests that, due to the symptoms of diabetes or other comorbid conditions common in type 2 diabetes mellitus (such as sleep apnoea), participants may be scoring higher on the PHQ-9 for symptoms which, for them, are not related to depression. This supports previous research, from other analysis of the SOUL-D cohort, which indicates that when the PHQ-9 is used in a population with type 2 diabetes mellitus a higher threshold or cut-off score to determine depression may be justified (Twist et al., 2013). As the cytokine factors were similarly correlated with both PHQ-9 depressive symptom clusters and the two PHQ-9 factors were very closely correlated it was decided to continue to use the PHQ-9 score as a total score in later analyses.

It was expected that the statistical significance of some of the associations reported in Chapter 3 may not be apparent in these analyses due the smaller sample sizes in this chapter and the resultant reduction in power. However, it was unexpected that some inflammatory markers that were not significantly associated with depressive symptom score in Chapter 3 would be significantly associated in this smaller sub-group, as was the case for IL-4. These differences suggest that a larger proportion of participants that were excluded from these analyses were coincidentally suffering from depressive symptoms less correlated with IL-4. That the association between depressive symptoms and IL-4 appears to occur in a significant proportion of the sample population here but in a lower

proportion of those excluded from the analyses in this chapter provided fits with the hypothesis of biological heterogeneity of the pathogenesis of depression.

In conclusion, one of the composite scores of inflammation that was created explains more of the variance in depression symptom score in this sample of newly diagnosed type 2 diabetes mellitus patients. This score may be a useful measure when evaluating associations between depressive symptom score and systemic inflammation in this group. The score appears to well represent the overall inflammatory state of the participants in this cohort and will be a useful tool when further analysing the relationship between depression and inflammation.

The next chapter will examine whether depression symptom score at diagnosis of diabetes is associated with concentrations of circulating inflammatory markers and change in inflammatory markers at follow-up. Future chapters will examine whether the concentrations of inflammatory markers at diagnosis of diabetes predict the later development of depressive symptomology.

Chapter 5: The Effects of Depression on Inflammatory Markers at 12-month Follow-up

Synopsis

Previous chapters have explored the cross-sectional association between depression and inflammation in a newly diagnosed type 2 diabetes mellitus cohort. The aim of this chapter is to examine the longitudinal association between depressive symptoms at baseline and systemic inflammation at 12-month follow-up. The hypothesis that depressive symptoms at baseline are associated with systemic inflammation after 12 months was tested. The independent association between depressive symptom score and each inflammatory marker was estimated by multiple linear regression, covariates adjusted for included: socio-demographic factors, adiposity, macrovascular disease, HbA_{1c}, prescribed medication, stressful life events. The secondary hypothesis tested was that concentrations of inflammatory markers do not improve in participants that were no longer symptomatic for depression at follow-up (determined by the PHQ-9), compared to those that were. Analysis of covariance (ANCOVA) was used to make comparisons between participants that were still depression cases after 12 months, compared to those that had improved symptoms and became non-depressed subjects. The main results in this chapter were that a higher PHQ-9 score at baseline was still associated with increased inflammation at follow-up after adjusting for relevant confounders, adjusting for the differences in inflammation at baseline and after accounting for changes in PHQ-9 score. These findings suggest that in those with comorbid depression increased systemic inflammation persists 12 months after diagnosis of type 2 diabetes mellitus. This inflammation may explain some of the increased complications and the poorer prognosis observed in this group.

Introduction

As was discussed in Chapter 1, depression is associated with increased inflammation in chronic disease populations of rheumatoid arthritis, cancers, type 2 diabetes mellitus and also in populations of otherwise healthy individuals (Dantzer et al., 2008). As was discussed in Chapter 1 and Chapter 3, because there is a bi-directional association between type 2 diabetes mellitus and depression, and existing studies on inflammation and depression in type 2 diabetes mellitus have been cross-sectional, determining whether inflammation is involved in both directions of this association has not been possible (Laake et al., 2014). This chapter investigates the longitudinal effects of depression on systemic inflammation in a cohort of newly diagnosed type 2 diabetes mellitus.

Those with type 2 diabetes mellitus and comorbid depression have significantly worse prognosis than those without (de Groot et al., 2001; Katon et al., 2005). It has been widely postulated that this is due to the negative impact of depression on diabetes self-care and glycaemic control in this group. However, when depression is treated successfully, glycaemic control generally does not improve (Ismail et al., 2007). This suggests that other biological factors, which are not resolved by current standard care for depression, may be involved in the increased morbidity and poorer prognosis in this group.

An alternative hypothesis is that increased basal inflammation may be the common antecedent of depression, type 2 diabetes mellitus and cardiovascular disease. Inflammation is associated with type 2 diabetes mellitus and may be involved in the pathogenesis of the condition (Pickup et al., 1997). It is also associated with insulin resistance and, as is the case in type 1 diabetes, may be involved in β-cell death (Donath and Halban, 2004). Increased inflammation is also associated with diabetes complications such as cardiovascular disease (Rajagopalan et al., 2001) and with depressive symptoms and depression in otherwise healthy individuals (Dowlati et al., 2010). Several mechanisms have

been proposed for the role of inflammation in the pathogenesis of depression, including altering permeability of the blood brain barrier and effecting dysfunction of the HPA axis.

In Chapter 3 and Chapter 4, cross-sectional associations between depressive symptoms and systemic inflammation were identified in a cohort of newly diagnosed type 2 diabetes patients (Laake et al., 2014). There are no longitudinal studies investigating inflammation and depression in cohorts of type 2 diabetes mellitus, however there are associations between systemic inflammation and the development of type 2 diabetes mellitus (Freeman et al., 2002; Pickup, 2004). Similarly, cross-sectional associations of depression and increased inflammation have been reported (Dowlati et al., 2010) and risk factors for depression (such as childhood adversity) have been associated with increased inflammation later in adult life (Hartwell et al., 2013). Individuals with comorbid depression and type 2 diabetes mellitus at diagnosis are also characterised by increased levels of systemic inflammation (Laake et al., 2014). If higher basal systemic inflammation is persistent in those with depression and type 2 diabetes mellitus, it may be involved in the pathogenesis of a faster progressing, more aggressive, sub-category of type 2 diabetes mellitus.

This chapter examines how inflammation changes over time in relation to depressive symptoms. The aims of this chapter are to identify whether the cross-sectional associations between depression and inflammation, reported at diagnosis of type 2 diabetes mellitus (Laake et al., 2014), exist throughout the course of type 2 diabetes mellitus. Specifically, to test the hypotheses that depressive symptoms at baseline result in increased inflammation throughout the course of type 2 diabetes mellitus and that reductions in depressive symptoms are not associated with reductions in concentrations of inflammatory markers.

Methods

Design, Setting, Sampling frame: The study design, setting and sampling frame have been previously described in the methods chapter (Chapter 2). Follow-up appointments were arranged 12 -months after baseline appointment, participants were sent reminder letters and contacted by telephone to arrange appointments. Non-responders were contacted at least three times over a four-month period before being classed as non-contactable.

Study population and case definition: The study population and case definition were the same as for the previous chapters (Chapter 3 & Chapter 4). Recruitment and baseline appointments for data presented in this chapter were conducted between May 2008 and September 2012. Follow-up appointments 12 months after baseline were conducted between August 2009 and October 2013 (Figure 5.1).

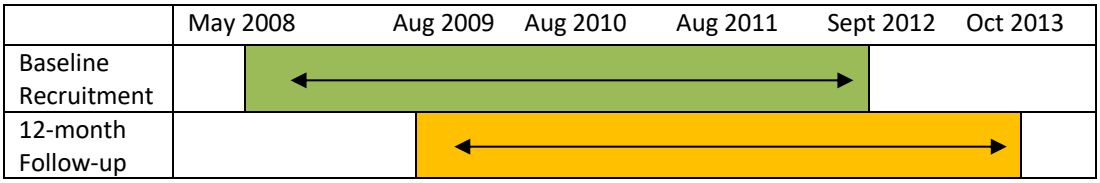


Figure 5.1 - Recruitment and follow-up appointment timeline.

Main explanatory variable: The presence of depressive symptoms at baseline and 12-month follow-up was measured using the PHQ-9. This is a self-report measure that was developed for primary care to aid clinicians in identifying probable cases of depression. A cut-off score of ≥ 10 is the optimal threshold for identifying probable cases of depression, with a sensitivity of 73% and specificity of 98 % (Spitzer et al., 1999). PHQ-9 scores ≥ 5, 10, 15, and 20 represented mild, moderate, moderately severe, and severe depression respectively (Kroenke et al., 2001). The PHQ-9 also is valid in type 2 diabetes mellitus populations. Although a cut-off score of ≥ 12 may be more appropriate for identifying probable cases of depression in this population (Twist et al., 2013) a cut of ≥ 10 has been used for

this chapter, so that results are can be compared to other studies. Full details on the collection of this data are presented in Chapter 2.

Main outcome variables: CRP was selected as the main inflammatory marker for follow-up. CRP has been identified *a priori* as associated with chronic conditions including type 2 diabetes mellitus and depression (Ridker et al., 2002; Pickup, 2004; Liukkonen et al., 2006; Fernandez-Real and Pickup, 2012; Lamers et al., 2013; Wium-Andersen et al., 2013). Serum CRP was measured by an hs-CRP assay from serum samples centrifuged from freshly drawn venous blood samples stored at room temperature until analysed. Fasting lipid tests were carried out using freshly drawn venous blood samples stored at room temperature until analysed. Detailed methods are provided in full in the methods (Chapter 2).

Assessment of confounders: Confounders included were the same as those in Chapter 3 and Chapter 4: age, gender, history of macrovascular disease (MI, CABG, CVA, carotid or limb re-vascularisation), current prescribed medications with a possible anti-inflammatory action (statins, fibrates, systemic steroids, non-steroidal anti-inflammatory drugs, COX-2 inhibitors), HbA_{1c}, body mass index (kg/m^2), smoking status and self-report ethnicity. Stressful life events were recorded at 12-month follow-up by self-report questionnaire. Detailed information on each variable and of their relevance as confounders has been previously outlined in Chapter 2.

Statistical Analyses: Data were analysed using SPSS 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics, Version 21.0. Armonk, NY). The main characteristics of the study population 12 months after baseline appointment are summarised as mean (SD) where data were normally distributed, median (IQR) where data were skewed, or as a count (percentage) for categorical variables, and are stratified by PHQ-9 depression case status at baseline. Unadjusted statistical analyses were conducted using Student's *t* test for normally distributed continuous data, Mann-

Whitney U analyses for non-normally distributed continuous data. The χ^2 test was used for comparisons of categorical data. A natural log was used to transform skewed data for inflammatory markers and PHQ-9 score in multiple regressions. For calculation of change in the concentration of CRP extreme outliers were excluded from the analysis, these included those with a change in CRP between baseline and follow-up of > 10 mg/L. These outliers were excluded as such extreme changes in CRP of more than 10 mg/L are most probably due to viral or bacterial infections or the development of other chronic inflammatory disease. These outliers were equally distributed across the four groups.

Multiple linear regressions were used to assess the relationship between depressive symptom score as the dependent variable and those inflammatory markers that had significant association in the unadjusted analyses as independent variables. Covariates were added to the model in sequential steps using a hierarchical method and only retained if they were significantly associated with the outcome or an important clinical confounder, such as ethnicity. Simes' improved Bonferroni method was used to correct for multiple testing for comparisons of inflammatory marker differences between groups and association between inflammatory marker concentrations and PHQ-9 score. An assessment of the residuals did not suggest major violations of the assumptions of a multiple regression. A sensitivity analysis showed there was no difference in the time between baseline appointment and 12-month follow-up between the two groups.

Two, two by two between-groups ANCOVAs were used to compare the effects of the interaction between change in baseline depression case status over 12 months and stressful life events on inflammation. The first (ANCOVA-A) for those that were depressed at baseline and the second (ANCOVA-B) for those that were not. ANCOVA is an adjusted form of analysis of variance (ANOVA) that allows comparison of variance between groups while controlling for additional variables. Initially multiple regression is used to isolate and remove variation of

the dependent variable that is explained by the covariates and then ANOVA is conducted on the adjusted values.

In ANCOVA-A the independent variables were change in depression case status (remained non-depressed, became depressed) and stressful life events over the 12-month monitoring period (one or more, none). In ANCOVA-B the independent variables were change in depression case status (remained depressed, became non-depressed), and stressful life events over the 12-month monitoring period (one or more, none). The dependent variable was CRP, measured at follow-up appointment (12-month). Values of age, gender, ethnicity, CRP, HbA1c, BMI, macrovascular disease and the prescription of medication measured at baseline were adjusted for as covariates in the analyses.

The assumptions for ANCOVA were met including equality of variance (Levene's Test, $p > 0.05$) and homogeneity of regression for the covariate and the dependent variable (which was similar between the two PHQ-9 groups in both analyses). There was not independence of the covariates and PHQ-9 groups. However, violation of this assumption of ANCOVA is accepted in observational studies.

Results

Of the 1790 participants with newly diagnosed type 2 diabetes mellitus recruited at baseline (Chapter 3), 1573 (87.9%) were seen for 12-month follow-up appointments or data was collected from their GP surgery from their annual diabetes health check. There was no significant difference between the number of participants lost to follow-up between the groups that were depressed and non-depressed at baseline. There were > 25% missing answers from PHQ-9 scores in 17 (0.9%) of the participants at baseline, these were excluded and the analyses were conducted on n = 1556 (86.9%). Longitudinal analyses of CRP were conducted on a subset of the cohort n = 1020 (57.7 %) for whom a serum sample for analysis was also obtained at baseline and 12-month follow-up and who had a > 25% missing answers from PHQ-9 score at follow-up (Table 5.1).

Table 5.1: Baseline characteristics of participants with type 2 diabetes mellitus in the South London Diabetes Study with a PHQ-9 score at baseline stratified by whether there was a frozen serum sample at follow-up for subjects

Baseline variable	Total (n = 1769)	Analyses Group (n = 1020)	Non Analyses Group (n = 749)	p-value
Mean age, years	56.1 (11.04)	57.33 (10.59)	54.41 (11.40)	<0.001*
Gender (%)				
Male	976 (55.2)	567 (55.6)	409 (54.6)	0.168
Female	793 (44.8)	453 (44.4)	340 (45.4)	
Ethnicity (%)				
White	878 (49.6)	545 (53.4)	333 (44.5)	<0.001*
Black	710 (40.1)	372 (36.5)	338 (45.1)	
Asian/Other	181 (10.2)	103 (10.1)	78 (10.4)	
HbA_{1c}				
Mean % HbA_{1c}	7.00 (1.45)	6.95 (1.36)	7.09 (1.58)	0.068
Mean HbA_{1c}, mmol/mol	53.1 (15.9)	52.5 (14.9)	53.9 (17.3)	
Psychological assessment				
Median PHQ-9 score	3.0 (0.0-6.0)	2.0 (0.0-6.0)	3.0 (0.0-7.0)	0.324
PHQ-9 depression (%)	258 (14.6)	139 (13.6)	119 (15.9)	0.183

***significant α=0.05. T-test and Mann Witney U test used for normal and non-normal data**

Table 5.2 reports the demographic characteristics and concentrations of CRP in the type 2 diabetes patients at the 12-month follow-up, stratified by PHQ-9 depression status at baseline. The prevalence of depression cases at baseline in this sample, defined as a PHQ-9 score of ≥ 10, was 14.1% (n = 220). After 12 months baseline depression cases had significantly higher BMIs and higher prevalence of macrovascular disease. After 12 months median circulating concentrations of CRP were significantly higher in the baseline depression cases compared to those that were non-depressed at baseline. At the 12-month follow-up, median concentrations of TGL (a proxy marker of inflammation) were also higher in the baseline depression cases. These differences remained statistically significant after Simes' improved Bonferroni correction for multiple testing. There was no statistically significant difference in mean HbA_{1c} after 12 months between depression cases and non-depressed subjects at baseline.

Table 5.3 reports the change (Δ) in HbA_{1c}, BMI, inflammatory markers, PHQ-9 score and the incidence of macrovascular disease between baseline and 12-month follow-up appointment, stratified by PHQ-9 depression case status at baseline for the 1020 participants included in the regressions and ANCOVA. After 12 months baseline depression cases had a significantly reduced PHQ-9 score compared to non-depressed subjects. There was no statistically significant difference in the change in HbA_{1c}, BMI, or inflammatory markers or in the diagnosis of macrovascular disease between the two groups.

Table 5.2: Characteristics of participants with type 2 diabetes mellitus in the South London Diabetes Study at baseline and 12 months after diagnosis stratified by baseline PHQ-9 depression status

Variable	Total (n = 1556)	No depression at baseline (n = 1336)	Depression at baseline (n = 220)	p-value
Baseline mean age, years	56.5 (10.95)	57.0 (11.02)	53.35 (9.98)	<0.001*
Gender (%)				
Male	857 (55.1)	753 (56.4)	104 (47.3)	0.012*
Female	699 (44.9)	583 (43.6)	116 (52.7)	
Ethnicity (%)				
White	793 (51.0)	684 (51.2)	109 (49.5)	0.062
Black	605 (38.9)	526 (39.3)	79 (35.6)	
Asian/Other	158 (10.2)	126 (9.4)	32 (14.5)	
Mean HbA_{1c}				
Baseline				
% HbA_{1c}	6.98 (1.40)	6.95 (1.40)	7.13 (1.42)	0.085
HbA_{1c}, mmol/mol	52.8 (15.3)	52.5 (15.3)	54.5 (15.6)	
12 month				
% HbA_{1c}	6.90 (1.26)	6.89 (1.26)	6.97 (1.28)	0.357
HbA_{1c}, mmol/mol	51.9 (13.8)	51.8 (13.8)	52.7 (14.0)	
Body mass index				
Baseline				
Mean BMI, kg/m²	31.9 (6.40)	31.7 (6.29)	33.2 (6.94)	0.004*
12 month				
Mean BMI, kg/m²	31.9 (6.24)	31.7 (6.20)	32.9 (6.41)	0.007*
Macrovascular disease (%)				
Baseline				
None	1391 (90.5)	1204 (91.3)	186 (85.7)	0.010*
More than 1	146 (9.5)	115 (8.7)	31 (14.3)	
12-month				
None	1302 (88.6)	1129 (89.5)	173 (82.8)	0.004*
More than 1	168 (11.4)	132 (10.5)	36 (17.2)	
Inflammatory marker				
Baseline				
Median CRP, mg/L	2.80 (1.20-6.40)	2.70 (1.20-6.20)	3.15 (1.40-8.65)	0.012*
12-Month				
Median CRP, mg/L	2.30 (1.00-5.30)	2.20 (0.98-5.20)	3.10 (1.38-6.85)	<0.001*
Baseline				
Median TGL, mmol/L	1.40 (0.93-2.00)	1.40 (0.90-1.90)	1.50 (1.10-2.30)	<0.001*
12-Month				
Median TGL, mmol/L	1.40 (0.90-2.00)	1.30 (0.90-1.90)	1.50 (1.00-2.23)	0.027*

*** significant after Simes' improved Bonferroni correction for multiple testing**

Table 5.3: Change (Δ) in characteristics of participants with type 2 diabetes mellitus in the South London Diabetes Study between baseline and 12 months follow-up, stratified by baseline PHQ-9 depression status.

Variable	Total (n = 1020)	Non-depressed subject at baseline (n = 881)	Depression case subject at baseline (n = 139)	p-value
Mean Δ % HbA_{1c}	-0.10 (1.26)	-0.09 (1.24)	-0.17 (1.34)	0.486
Median *ΔBMI, kg/m²*	0.02 (-1.00-1.10)	0.10 (-0.98-1.10)	0.00 (-1.37-1.01)	0.185
New macrovascular event (%)	17 (1.7)	15 (1.7)	2 (1.4)	0.823
Median *Δ TGL, mmol/L*	0.00 (-0.3-0.3)	0.00 (-0.3-0.3)	-0.10 (-0.4-0.30)	0.083
Median *Δ CRP, mg/L*	0.20 (-1.70-0.60)	-0.20 (-1.80-0.60)	-0.10 (-1.60-0.80)	0.215
Mean *Δ PHQ-9 Score*	-0.47 (4.34)	0.28 (3.45)	-5.00 (6.11)	<0.001*

*** significant after Simes' improved Bonferroni correction for multiple testing**

Table 5.4 reports the associations of depressive symptoms, measured as a continuous PHQ-9 score (0-27), at baseline and inflammatory markers after 12 months while adjusting for covariates (socio-demographic factors, HbA_{1c}, adiposity (BMI), smoking, history of macrovascular disease and prescription of medication). After adjusting for all covariates there remained a significant association between symptoms of depression, and CRP but not TGL. Table 5.5 reports the associations of depressive symptoms at baseline and inflammatory markers after 12 months after adjusting for baseline inflammation. After adjusting for all covariates there remained a significant association between symptoms of depression, and CRP but not TGL. These differences became a non-significant trend after Simes' improved Bonferroni correction for multiple testing.

Table 5.4: Adjusted[†] final multiple linear regression models for the independent association between two inflammatory markers at 12-month follow-up[‡] and PHQ-9 score at baseline[‡] n = 1020

Model	R^2		PHQ-9	Age	Gender	Ethnicity	HbA1c	BMI	MVD	Smoking
CRP	0.19	B (SE)	0.07 (0.02)	-0.001 (0.002)	0.15 (0.05)	-0.14 (0.05)	0.006 (0.002)	0.04 (0.004)	0.11 (0.08)	0.16 (0.56)
		standardised-b	0.09	-0.01	0.10	-0.09	0.11	0.34	0.04	0.09
		p	0.005*	0.821	0.001*	0.003*	<0.001*	<0.001*	0.163	0.004*
TGL	0.16	B (SE)	0.01 (0.01)	-0.003 (0.001)	-0.06 (0.02)	-0.23 (0.02)	0.001 (0.001)	0.004 (0.002)	-0.02 (0.04)	0.13 (0.03)
		standardised-b	0.03	-0.10	-0.08	-0.32	0.04	0.06	-0.02	0.15
		p	0.383	0.003*	0.013*	<0.001	0.253	0.042	0.644	<0.001

[*]significant after Simes' improved Bonferroni correction for multiple testing. [†]Adjusted for age, gender, ethnicity, HbA_{1c}, BMI, smoking, history of macrovascular disease (MVD) and prescribed medications at baseline. Only age, gender, ethnicity, HbA1c, BMI MVD and smoking are displayed here as these were the explanatory covariates. [‡]These variables were ln transformed

Table 5.5: Adjusted[†] final multiple linear regression models for the independent association between inflammatory markers at 12-month follow-up[‡] and PHQ-9 score at baseline[‡], after adjusting for baseline inflammation[‡] n = 1020

Model	R^2		PHQ-9	Marker at Baseline	Gender	Ethnicity	BMI	MVD
CRP	0.47	B (SE)	0.04 (0.02)	0.53 (0.02)	0.07 (0.04)	-0.08 (0.04)	0.017 (0.003)	0.10 (0706)
		standardised-b	0.05	0.58	0.05	-0.05	0.14	0.04
		p	0.036	<0.001*	0.056	0.052	<0.001*	0.121
TGL	0.62	B (SE)	-0.002 (0.008)	0.79 (0.02)	-0.07 (0.02)	-0.07 (0.02)	0.001 (0.001)	-0.05 (0.03)
		standardised-b	-0.005	0.75	-0.01	-0.10	0.01	-0.04
		p	0.828	<0.001*	0.640	<0.001*	0.722	0.068

[*]significant after Simes' improved Bonferroni correction for multiple testing. [†]Adjusted for markers at baseline, age, gender, ethnicity, HbA_{1c}, BMI, smoking, history of macrovascular disease (MVD) and prescribed medications. Only marker at baseline, age, gender, ethnicity, BMI and MVD are displayed here as these were the explanatory covariates. [‡]these variables were ln transformed

Figure 5.2: Graph to show unadjusted change in CRP depending on PHQ-9 depression case status at baseline and 12 month follow-up (*n* =1020)

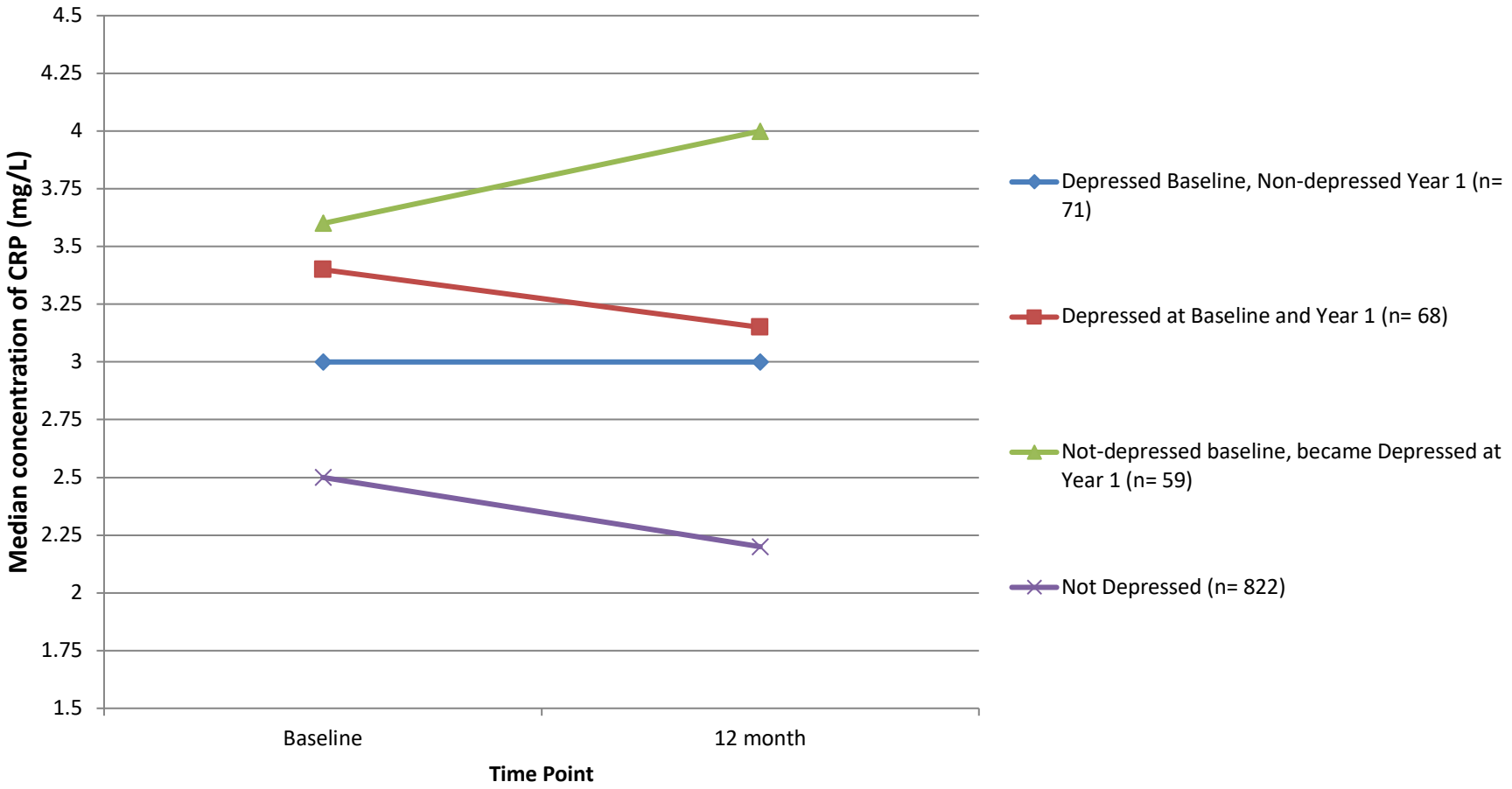


Figure 5.2 presents the median CRP concentrations of CRP at baseline and 12-month follow-up for each of the four PHQ-9 depression case groups. All groups containing depression cases at baseline or 12-month follow-up had a median concentration of CRP ≥ 3 mg/L. While those that were non-depressed subjects at baseline and 12-month follow-up had median concentrations of CRP ≤ 2.5 mg/L.

Two ANCOVAs were conducted to identify differences between four different groups to identify effects of any interaction between stressful life events and change in depression status on CRP concentration at follow-up after adjusting for covariates.

Depression Status Categories:

1. Depression case subject at baseline and at 12-month follow-up
2. Depression case subject at baseline but non-depressed subject at 12-month follow-up
3. Non-depressed subject at baseline but depression case subject at 12-month follow-up
4. Non-depressed subject at baseline and at 12-month follow-up.

Stressful Life Events Categories:

1. 1 or more major stressful life events between baseline and 12-month follow-up
2. No major stressful life events between baseline and 12-month follow-up

After adjusting for covariates, there was no significant interaction effect between stressful life events and change in depression status and CRP. For the difference between those that were non-depressed subjects vs. those that became depression cases change in depression status (one of the main effects) was statistically significant ($p = 0.002$), with a small effect size (partial eta squared = 0.01), however stressful life events were not statistically significant. For ANCOVA-b change in depression status effect was not statistically significant,

however stressful life events effect was statistically significant (p = 0.045), with a moderate effect size (partial eta squared = 0.04).

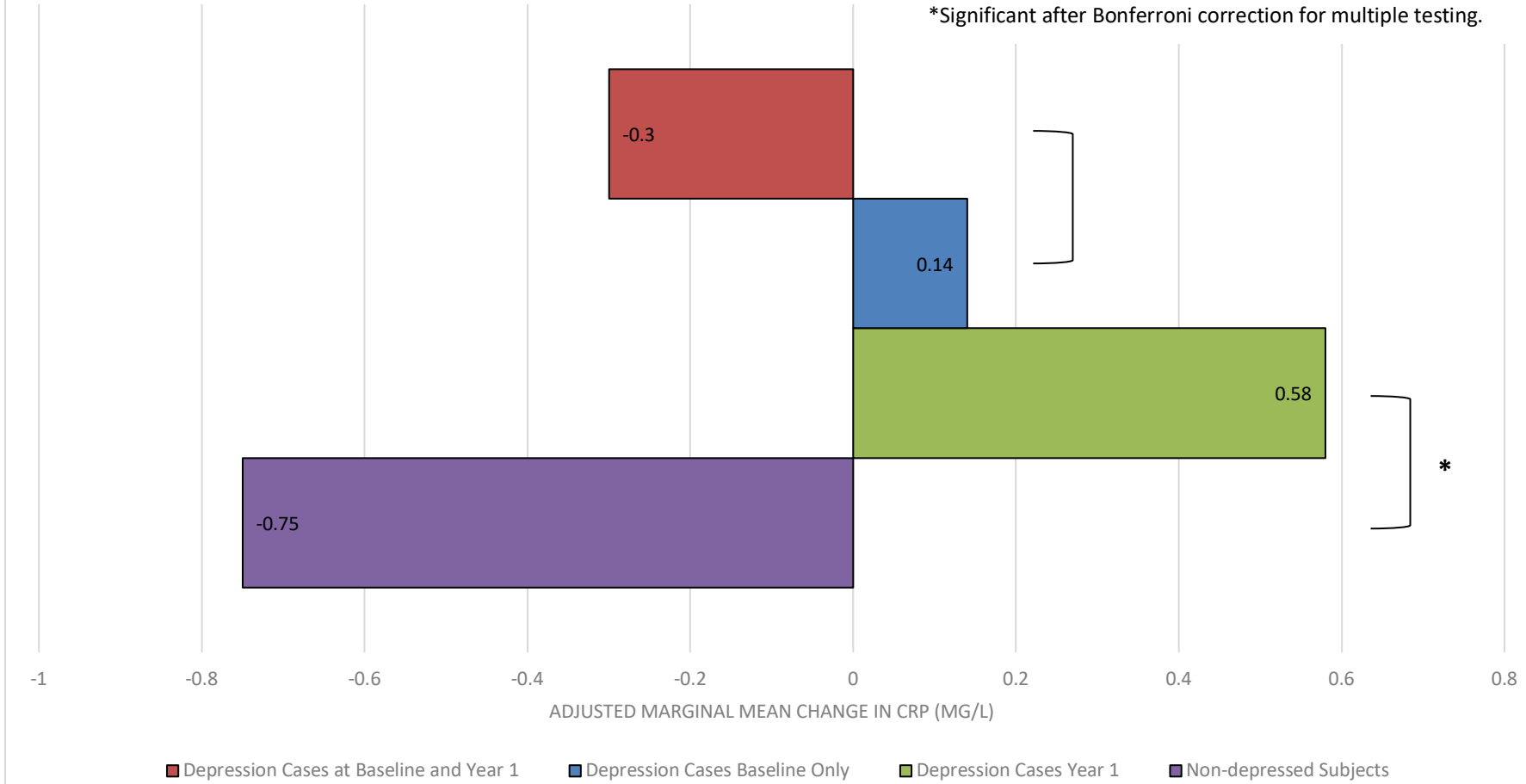
Table 5.6. Adjusted† Marginal Means from ANCOVA models for change in mean CRP at 12-month follow-up stratified by PHQ-9 depression status at baseline and 12-month follow-up

PHQ-9 status (baseline and 12-month follow-up)	Adjusted Marginal Mean change in CRP (mg/L)	Mean Difference in change in CRP (mg/L)	p-value
ANCOVA A			
Non-depressed subjects	-0.75	1.327	0.002*
Depression cases at year 1	0.58		
ANCOVA B			
Depression cases at baseline only	0.14	0.445	0.238
Depression cases at baseline and year 1	-0.30		

†Adjusted for baseline age, gender, ethnicity, HbA1c, macrovascular disease, smoking, prescription of medication BMI and any stressful life events between baseline and 12-month follow-up. *Significant after Bonferroni correction for multiple testing.

Table 5.6 and figure 5.3 report the adjusted marginal means from the ANCOVAs. There was a statistically significant difference in adjusted mean CRP concentrations between non-depressed subjects (at baseline and 12-month follow-up) and those who became depression cases at 12-month follow-up. There was no statistically significant difference in adjusted mean CRP concentration between depression cases at baseline who became non-depressed subjects at 12-month follow-up and those that remained depression cases.

Figure 5.3: Graph to show adjusted marginal means from ANCOVA A and ANCOVA B



Discussion

Twelve months after baseline, in a large population-based cohort of newly diagnosed type-2 diabetes mellitus, those that were depression cases at baseline had significantly higher concentrations of serum CRP than those that were non-depressed subjects at baseline. After adjusting for relevant potential confounding variables, including, adiposity, age, gender, ethnicity, smoking, HbA_{1c}, macrovascular disease and medications with anti-inflammatory action, and adjusting for the CRP at baseline, there was still a non-significant trend for a weak association between depressive symptoms at baseline and serum CRP at 12-month follow-up. At 12-month follow-up, those that had been depression cases at baseline or 12-month follow-up had significantly higher concentrations of CRP than those that were never depression cases. There was no difference in glycaemic control or inflammation between those participants who had improved PHQ-9 depressive symptoms to a sub-threshold level and those that remained depression cases.

One strength of the analyses in this chapter is that data were collected using a population-based prospective cohort study design. This aims to reduce selection bias and allows for identification of the longitudinal associations described while adjusting for a range of relevant covariates, including stressful life events between baseline and 12 months. As these type 2 diabetes patients were newly diagnosed at recruitment (less than 18 months duration of diabetes at 12-month follow-up) only 55 (4%) were on insulin therapy at follow-up, which was equally prescribed in those who were depression cases versus those who were not, otherwise insulin may have been a significant source of confounding. The inflammatory marker studied, CRP, is used in routine clinical practice is a component of the acute phase response in animal models and humans and may have clinical applications in the future as a useful biomarker in this area. Use of the PHQ-9 score allows for both categorical binary comparisons between depression and non-depressed subjects and, for improved power, regressions to

be carried out to identify and associations between number of depressive symptoms and concentrations of inflammatory markers.

The main limitation of these analyses is that depression cases were classified using the PHQ-9 cut-off score, and a clinical diagnosis of depression was not used. As the PHQ-9 cut-off of ≥ 10 has a lower specificity than sensitivity, (Twist et al., 2013) there may be some participants classified as depression cases that were not suffering from major depression. Antidepressants and psychotherapy were not adjusted for, but there is no systematic evidence from randomised control trials or observation studies to support a direct pro- or anti-inflammatory effect of antidepressants or psychotherapy. It is possible that one of these two treatments would have been associated with a reduction in inflammation. Additionally, 43% of the sample population were excluded from the analyses because of missing data. Subjects that had missing data were significantly younger, and so the associations described here may be less applicable to the younger newly diagnosed type 2 diabetes mellitus population. A further limitation is that only one inflammatory marker was measured at the 12-month follow-up, but it is a marker that is consistently found to be associated with depression and type 2 diabetes mellitus. There is a further risk of residual confounding for poor adherence to medication, which it was not possible to measure, although medication data was derived from current GP prescription records. There is a small risk that comorbid acute or chronic inflammatory conditions may have been included in the cohort, which could have introduced a significant source of error, but terminal and advanced conditions were exclusion criteria so the more severely affected cases were excluded. Oestrogen replacement therapy and any potential inflammatory effects of pre-menopause were not adjusted for but the majority of females in the study population were of a post-menopausal age. Diet and physical activity were not included because self-report measures are not sufficiently accurate, but BMI was used and may be considered a proxy marker for both. In a clinical setting generally a 50% reduction in depressive symptoms is considered significant for trials testing antidepressant therapies.

As the definition of a depression case subject at baseline and year 1 was a PHQ-9 score of ≥ 10 in these analyses, there is the possibility that some participants included in the groups for a change in depression status may only have had a small change in actual symptoms between the two time-points. Approximately 75% of the group who were classed as depression cases at baseline and non-depressed subjects at follow-up had a ≥ 6-point improvement in PHQ-9 score, therefore the majority of this group did have a clinically relevant improvement in symptoms. Approximately 50% of the group that were depression cases at 12-month follow-up but were not at baseline had a ≥ 6-point increase in PHQ-9 score. The majority of these participants had PHQ-9 score of ≥ 5 at baseline, which is indicative of mild depression. Comparatively more than 75% of the group who were non-depressed subjects at baseline and 12-month follow-up had a PHQ-9 score of < 5. An additional limitation is that only 12-month follow-up data was available for this analysis, therefore any associations of inflammation with future late diabetes complications in this population cannot yet be examined.

In type 2 diabetes mellitus, depression is significantly associated with increased risk of complications and a poorer prognosis (de Groot et al., 2001). It has been suggested that this may be due to impaired self-care behaviours and poorer glucose control (elevated HbA1c) that others have observed in this group (Ciechanowski et al., 2000; Lustman et al., 2000). However, in this analysis there were no significant differences in HbA_{1c} between baseline depression cases and non-depressed subjects 12 months after baseline (12-18 months after diagnosis). This is consistent with the literature, as glycaemic control does not generally improve when depression alone is treated (Ismail et al., 2007), and adds weight to the hypothesis that the accelerated progression of diabetes complications observed in depression and comorbid type 2 diabetes mellitus are not mediated by reduced diabetes self-care behaviours alone.

Serum CRP, positively associated with baseline depressive symptom score in this cohort at baseline (Chapter 3) and 12-month follow-up, is an acute phase reactant that is used in routine clinical practice to identify infection. It is also used as a biomarker for risk of cardiovascular disease with concentrations of > 2mg/L and > 3mg/L signifying medium and high risk (table 5.7).

Table 5.7. CRP concentration as a risk factor for cardiovascular disease risk

CRP	> 1 mg/L	> 2 mg/L	> 3 mg/L
Cardiovascular disease risk	Low	Medium	High

In this cohort, baseline depression cases have significantly higher median concentrations of serum CRP at the 12-month follow-up. The median CRP for baseline depression cases is indicative of high risk of cardiovascular disease (as it was at baseline). In comparison the median in the non-depressed group is indicative of medium risk of cardiovascular disease. Although they have similar glycaemic control, at 12-month follow-up the baseline depression cases remain at higher risk of cardiovascular disease than non-depressed subjects. That depression symptom score at baseline is associated with a persistent increase in concentrations of CRP after adjusting for covariates (such as glycaemic control) suggests that worse glycaemic control is not responsible for the worse prognosis of patients with comorbid depression and type 2 diabetes mellitus.

Of the participants who were depressed at baseline, those who had improved depressive symptoms at 12-month follow-up, and were classed (according to the PHQ-9) as having sub-threshold depression or being non-symptomatic, had similar concentrations of CRP to those participants who were still depressed. This supports the hypothesis that a significant proportion of the increased inflammation in this group is not as a result of altered or poorer diabetes self-care behaviours in those with depression, instead it is likely to be a persistent

biological characteristic of this group of patients that does not improve with the resolution of depressive symptoms.

To conclude, the associations reported in this chapter support the hypothesis that depression in type 2 diabetes mellitus is characterised by increased basal systemic inflammation that persists even when depressive symptoms are improved. Future work should identify whether any current anti-inflammatory treatments successfully improve depressive symptoms and diabetes complications in this group. As a longitudinal association between depressive symptoms and an inflammatory marker (CRP) has been established in this chapter, the next chapter will examine whether increased systemic inflammation at diagnosis with type 2 diabetes mellitus is associated with the incidence of depression or depression symptoms in the following 12 months.

The majority of participants who became depression cases at the 12-month follow-up had mild or sub-threshold depression at baseline and also had a significantly higher concentration of CRP at baseline when compared to participants who did not go on to become depression cases. The next chapter will examine whether, after adjusting for covariates, there is a significant association between systemic inflammation at baseline and change in depressive symptoms 12 months later.

Chapter 6: Inflammation and the Prevalence of Depression at 12-month Follow-up

Synopsis

Previous chapters have explored the relationship between depressive symptoms with inflammation later in the course of type 2 diabetes mellitus. The aim of this chapter is to examine the relationship between increased inflammation at diagnosis with type 2 diabetes mellitus, its association with depressive symptoms and the incidence in depressive symptoms in the subsequent 12 months. The hypothesis that increased systemic inflammation at baseline is associated with increased prevalence of depression incidence at 12-month follow-up was tested. The increased prevalence in depression incidence was measured by Chi^2 test. A subsidiary hypothesis was that systemic inflammation at baseline was associated with PHQ-9 score and change in PHQ-9 score 12 months later; this was measured by unadjusted Spearman's ranked correlations. Multiple linear regressions were used to adjust for covariates. Covariates adjusted for included, socio-demographic factors, adiposity, macrovascular disease, HbA_{1c}, prescribed medication, stressful life events and the baseline PHQ-9 score. The main results were that inflammation at baseline was associated with PHQ-9 score at 12-month follow-up and after adjusting for covariates this association remained, however, after adjusting for baseline PHQ-9 score this association was attenuated. This suggests that, at this early stage of type 2 diabetes mellitus, inflammation does not cause a significant change in depressive symptoms 12 months later.

Introduction

The literature review in Chapter 1 describes the bi-directional association between depression and type 2 diabetes mellitus and the possible role of inflammation in both the increased incidence of diabetes complications observed in depression, and in the increased incidence of depression observed in type 2 diabetes mellitus. Chapter 3, Chapter 4 and Chapter 5 have examined the association between depression at baseline and inflammation over time in a newly diagnosed type 2 diabetes cohort. PHQ-9 score is associated with increased systemic inflammation at baseline and the increased inflammation in depression cases persists after 12 months even after adjusting for change in depression status. This chapter will address whether inflammation at baseline is associated with an increased incidence of PHQ-9 depression.

Several meta-analyses describe an increased risk of depression in type 2 diabetes mellitus, which ranges between 15-24% (Mezuk et al., 2008; Nouwen et al., 2010). Some of this increased prevalence of depression may be due to the difficult psychological burden of living and managing a chronic condition with complications (Winkley, 2010). As there is a cross-sectional association of depression and late diabetes complications (Pouwer et al., 2003) some of the increased incidence may also be due to the stress of dealing with these painful and disabling conditions. An alternative or additional explanatory variable may be increased systemic inflammation, which may be the common antecedent for type 2 diabetes mellitus, and some of the additional cases of depression.

The inflammatory hypothesis is one of several proposed models for the pathogenesis of depression, which are described in detail in Chapter 1. It is likely that the pathogenesis of depression is heterogeneous but that some forms of depression have an inflammatory pathogenesis. Inflammation may be an effector of the HPA axis dysfunction commonly seen in depression (Stetler and Miller, 2011).

Inflammation as a risk factor for depression has been well studied in hepatitis-C and cancer where interferon (IFN)-α (a pro-inflammatory cytokine with anti-viral and anti-proliferative properties) is used as a treatment. IFN-α induces increases in inflammatory biomarkers and is associated with an increase of depression incidence by as much as 50% (Capuron et al., 2002). Longitudinal studies have reported that in those receiving IFN- α treatment exhibit changes in systemic concentrations of endogenous cytokines which are greater in those that develop depression compared to those that do not (Capuron and Miller, 2004). Depressive symptoms of this cytokine-induced depression are similar to idiopathic depression and are improved by the SSRI paroxetine (Capuron et al., 2002).

Inflammation may also be a risk factor for, and be involved in, the pathogenesis of idiopathic depression. The majority of studies investigating the association of inflammation and depression have been cross-sectional and so the direction of the association can only be inferred. There is evidence that childhood risk factors for depression are associated with long-term physiological differences later in life. For example, childhood adversity is associated with increased inflammation in adult life in those that do not suffer from depression (Hartwell et al., 2013). In addition, other risk factors for depression have short-term physiological effects of such as stressful life events, which are associated with increased NF-κβ (Bierhaus et al., 2004), IL-6, IL-1β and CRP expression (Steptoe et al., 2007).

As is discussed in Chapter 1 there is an increased incidence of depression in those with many chronic diseases including type 2 diabetes mellitus (Mezuk et al., 2008; Nouwen et al., 2010). As type 2 diabetes mellitus has a proposed inflammatory pathogenesis (Pickup et al., 1997) this chapter examines the hypothesis that some of the increased incidence of depression observed in the condition is explained by increased systemic inflammation prior to depression.

Methods

Design, Setting, Sampling frame: The study design, setting and sampling frame have been previously described in the methods chapter (Chapter 2).

Study population and case definition: The study population and case definition were the same as for the previous chapter (Chapter 3, Chapter 4 and Chapter 5). Recruitment and baseline appointments for data presented in this chapter were conducted between May 2008 and September 2012. Follow-up appointments 12 months after baseline were conducted between August 2009 and October 2013.

Main explanatory variables: Twelve markers of inflammation which had been identified *a priori* as associated with type 2 diabetes mellitus and/or depression (CRP, IL-4, IL-6, IL-10, VEGF, TNF-α, IL-1β, IL-1RA, MCP-1, WBC, TGL and Adiponectin) were included in the analysis. Full details on the collection of this data are presented in Chapter 2. The factor structure of the inflammatory markers and PHQ-9 score at baseline were determined by maximum likelihood factor analysis (Chapter 4). From this, composite scores were created from the sum of z-scores of the markers in each biomarker cluster. The composite score of CRP, IL-1RA, WBC, MCP-1, VEGF and TGL (Cytokine Index 2) was also used as a measure of baseline inflammation as it was more closely correlated with the PHQ-9 score at baseline than the individual markers it is referred to as 'inflammation score'.

Main outcome variable: The presence of depressive symptoms at baseline and 12-month follow-up was measured using the PHQ-9. This is a self-report measure that was developed for primary care to aid clinicians in identifying probable cases of depression. A cut-off score of ≥ 10 is the optimal threshold for identifying probable cases of depression, with a sensitivity of 73% and specificity of 98 % (Spitzer et al., 1999). PHQ-9 scores ≥ 5, 10, 15, and 20 represented mild,

moderate, moderately severe, and severe depression respectively (Kroenke et al., 2001). The PHQ-9 also is valid in type 2 diabetes mellitus populations. Although a cut-off score of ≥ 12 may be more appropriate for identifying probable cases of depression in this population (Twist et al., 2013) a cut of ≥ 10 has been used for this chapter, the rationale for this is discussed in Chapter 2 and Chapter 3. Full details on the collection of this data are presented in Chapter 2.

Assessment of confounders: Confounders included were the same as those in Chapter 5: age, gender, history of macrovascular disease (MI, CABG, CVA, carotid or limb re-vascularisation), current prescribed medications with a possible anti-inflammatory action (statins, fibrates, systemic steroids, non-steroidal anti-inflammatory drugs, COX-2 inhibitors), HbA_{1c}, body mass index (kg/m^2), smoking status, self-report ethnicity and stressful life events. Detailed information on each variable and of their relevance as confounders has been previously described in Chapter 2.

Statistical Analyses: Data were analysed using SPSS 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics, Version 21.0. Armonk, NY). The main characteristics of the study population 12 months after baseline appointment are summarised as mean (SD) where data were normally distributed, median (IQR) where data were skewed, or as a count (percentage) for categorical variables, and are stratified by PHQ-9 depression case status at baseline. Unadjusted statistical analyses were conducted using Student's *t* test for normally distributed continuous data, Mann-Whitney U analyses for non-normally distributed continuous data. The χ^2 test was used for comparisons of categorical data. A natural log was used to transform skewed data for inflammatory markers and PHQ-9 score in multiple regressions. Multiple linear regressions were used to assess the relationship between depressive symptom score as the dependent variable and those inflammatory markers that had significant association in the unadjusted analyses as independent variables; covariates were added to the model in sequential steps using a hierarchical method and only retained if they were significantly

associated with the outcome or an important clinical confounder, such as ethnicity. For 30 subjects no answers were recorded to two of the questions within the stressful life events questionnaire (administered at 12-month follow-up) relating to offspring. If no answer was recorded to either question and answers were recorded to all other questions it was assumed the case subject had no offspring. These subjects were then grouped according to their answers to the remainder of the stressful life events questionnaire. For nine subjects there were one or more missing answers to the stressful life events questionnaire and no other stressful life events recorded so these subjects were excluded from the analyses. For five subjects there were one or more missing answers to the questionnaire but other stressful life events were recorded, these subjects were included in the one or more stressful life events category. Simes' improved Bonferroni method was used to correct for multiple testing for comparisons of inflammatory marker differences between groups and association between inflammatory marker concentrations and PHQ-9 score. An assessment of the residuals did not suggest major violations of the assumptions of a multiple regression.

Results

Of the 1790 participants with newly diagnosed type 2 diabetes mellitus recruited at baseline (Chapter 3), 1573 (87.9%) were seen for 12-month follow-up appointments or data was collected from their GP surgery from their annual diabetes health check. There were > 25% missing answers from PHQ-9 scores in 17 (0.9%) of the participants at baseline, and an additional 195 (10.9%) at 12-month follow-up*[1]. These were excluded and the analyses were conducted on n = 1361 (76.0%). Analyses of incidence of above threshold PHQ-9 depression cases were conducted on a subset of the sample n = 1174 (86.2%) that had a PHQ-9 score of < 10 at baseline (Table 6.1).

Table 6.1: Characteristics of participants with type 2 diabetes mellitus in the South London Diabetes Study who had a PHQ-9 score at baseline stratified by whether there was a PHQ-9 score at follow-up for subjects

Baseline variable	Total (n = 1769)	Complete PHQ-9 (n = 1361)	Missing PHQ-9 (n = 408)	p-value
Mean age, years	56.1 (11.04)	57.01 (11.74)	53.05 (11.45)	<0.001*
Gender (%)				
Male	976 (55.2)	766 (56.3)	221 (51.5)	0.087
Female	793 (44.8)	595 (43.7)	208 (8.5)	
Ethnicity (%)				
White	878 (49.6)	720 (52.9)	158 (38.7)	<0.001*
Black	710 (40.1)	506 (37.2)	204 (50.0)	
Asian/Other	181 (10.2)	135 (9.9)	46 (11.3)	
HbA_{1c}				
Mean % HbA_{1c}	7.00 (1.45)	6.96 (1.40)	7.15 (1.62)	0.043*
Mean HbA_{1c}, mmol/mol	53.1 (15.9)	52.6 (15.3)	54.7 (17.7)	
Psychological Assessment				
PHQ-9 Score	3.0 (0.0-6.0)	3.0 (0.0-6.0)	3.0 (0.0-8.0)	0.318
PHQ-9 depression (%)	258 (14.6)	187 (13.7)	71 (17.4)	0.066

***significant α=0.05. T-test and Mann Witney U test used for normal and non-normal data**

Table 6.2 reports the unadjusted associations between inflammatory marker concentration at baseline and depressive symptoms, measured as a continuous PHQ-9 score (0-27) after 12 months. Of the 12 markers of inflammation, five were significantly associated with the PHQ-9 depressive symptom score at follow up (CRP, VEGF IL-1RA, TGL, Adiponectin), as was the combined inflammation score. These associations remained significant after Simes' improved Bonferroni correction for multiple testing for the inflammation score, CRP, IL-1RA and TGL but not for VEGF and Adiponectin.

Table 6.2: Unadjusted Spearman's ranked correlation for association between inflammatory marker concentration at baseline and PHQ-9 score at 12-month follow-up

Inflammatory marker	r_s	p-value
Inflammation Score (Cytokine Index 2)	0.12	<0.001*
CRP	0.12	<0.001*
IL-4	0.05	0.097
IL-6	0.08	0.801
IL-10	0.03	0.397
VEGF	0.07	0.032
TNF-α	-0.001	0.987
IL-1β	0.03	0.345
IL-1RA	0.09	0.006*
MCP-1	0.06	0.053
WBC	0.05	0.085
TGL	0.10	<0.001*
Adiponectin	-0.06	0.028

***significant after Simes' improved Bonferroni correction for multiple testing**

Table 6.3 reports the association between inflammatory marker concentrations at baseline and PHQ-9 score at follow-up after adjusting for covariates (socio-demographic factors, baseline HbA_{1c}, baseline adiposity (BMI), baseline smoking status, baseline history of macrovascular disease, baseline prescription of medication and also the presence of stressful life events between baseline and 12-month follow-up). After adjusting for all covariates, Inflammation Score, CRP,

MCP-1 and TGL remained significantly associated with depressive symptom score. These differences remained significant after Simes' improved Bonferroni correction for multiple testing for Inflammation Score and MCP-1 but not for CRP or TGL. A sensitivity analysis was conducted with the baseline PHQ-9 depression cases excluded from the regressions. Removing these subjects significantly reduced the power of the analyses however the unadjusted correlations remained significant for Inflammation Score, CRP, TGL and the adjusted correlations remained for Inflammation Score and TGL.

Table 6.4 reports the association between inflammatory marker concentrations at baseline and PHQ-9 score at follow-up after adjusting for covariates and for PHQ-9 score at baseline. After adjusting for all covariates none of the inflammatory markers that were associated with depressive symptoms at baseline remained significantly associated with depressive symptom score at 12-month follow-up.

Table 6.5 reports the demographic characteristics and 12-month follow-up PHQ-9 depression status of the sample population that were not PHQ-9 depression cases at baseline, stratified by their baseline inflammation score. Those with a baseline inflammation score < the mean inflammation score were included in the 'low inflammation score' group and those > the mean inflammation score were included in the 'high inflammation score' group. Those with higher inflammation score were older, more likely to be of white ethnic background, had higher BMIs and an increased history of macrovascular disease. Between baseline and the 12-month follow-up there was a slight (2-3%) difference in the incidence of stressful life events but this was not statistically significant. There was also 29% more incidence of depression cases in the group with more systemic inflammation but this was not a statistically significant difference (due to the small overall number of new depression cases). There was no statistically significant difference in the mean HbA_{1c} at baseline of after 12 months between the groups with higher vs. lower inflammation at baseline.

Table 6.3: Adjusted[†] final multiple linear regression models for the independent association between PHQ-9 score[‡] at 12-month follow-up and each inflammatory marker[‡] at baseline

Model	R^2	Inflammatory marker		Age	Gender	BMI	MVD	SLE	Smoking
Inflammation Score[1]	0.10	B (SE)	0.03 (0.10)	-0.01 (0.003)	0.11 (0.07)	0.01 (0.005)	0.32 (0.11)	0.23 (0.07)	0.28 (0.09)
		standardised-b	0.11	-0.16	0.06	0.07	0.10	0.11	0.12
		p	0.007*	<0.001*	0.094	0.065	0.006*	0.001*	0.001*
CRP	0.09	B (SE)	0.08 (0.04)	-0.01 (0.003)	0.17(0.06)	0.01 (0.005)	0.38 (0.10)	0.24 (0.06)	0.30 (0.07)
		standardised-b	0.07	-0.13	0.09	0.05	0.12	0.12	0.12
		p	0.040	<0.001*	0.004*	0.117	<0.001*	<0.001*	<0.001*
VEGF	0.08	B (SE)	0.07 (0.05)	-0.01 (0.003)	0.12 (0.07)	0.01 (0.005)	0.36(0.11)	0.20 (0.07)	0.30 (0.08)
		standardised-b	0.05	-0.13	0.06	0.09	0.11	0.10	0.12
		p	0.152	<0.001*	0.064	0.007*	0.001*	0.002*	<0.001*
IL-1RA	0.08	B (SE)	0.06 (0.05)	-0.01 (0.003)	0.12 (0.07)	0.01 (0.005)	0.36 (0.11)	0.19 (0.07)	0.29 (0.08)
		standardised-b	0.04	-0.13	0.06	0.09	0.11	0.10	0.12
		p	0.269	<0.001*	0.072	0.014*	0.001*	0.003*	<0.001*
MCP-1[#]	0.09	B (SE)	0.15 (0.05)	-0.01 (0.003)	0.14 (0.07)	0.01 (0.005)	0.36 (0.11)	0.20 (0.07)	0.29 (0.08)
		standardised-b	0.10	-0.14	0.07	0.09	0.11	0.10	0.12
		p	0.005*	<0.001*	0.029	0.005*	0.001*	0.002*	<0.001*
TGL	0.08	B (SE)	0.20 (0.09)	-0.01 (0.003)	0.19 (0.06)	0.01 (0.005)	0.34 (0.10)	0.21 (0.06)	0.30 (0.07)
		standardised-b	0.07	-0.11	0.10	0.07	0.11	0.11	0.12
		p	0.026	0.001*	0.001*	0.014*	<0.001*	<0.001*	<0.001*
Adiponectin	0.08	B (SE)	-0.03 (0.05)	-0.01 (0.003)	0.20 (0.06)	0.01 (0.005)	0.39 (0.10)	0.22 (0.06)	0.30 (0.07)
		standardised-b	-0.02	-0.11	0.10	0.08	0.12	0.11	0.12
		p	0.520	<0.001*	0.002*	0.010*	<0.001*	<0.001*	<0.001*

[1] Composite score of the sum of the z-scores of CRP, IL-1RA, WBC, MCP-1, VEGF and TGL at baseline based on factor analysis (Chapter 4).

[*]significant after Simes' improved Bonferroni correction for multiple testing

[†]adjusted for age, gender, ethnicity, HbA_{1c}, BMI, smoking, history of macrovascular disease (MVD) and prescribed medications (baseline) and stressful life events between baseline and 12 months.

Only age, gender, BMI, MVD and Stressful Life Events (SLE) are displayed here as these were the explanatory covariates.

[‡]these variables were ln transformed

[#]MCP-1 was trending close to significance in the unadjusted Spearman's correlation and was associated with depression score at baseline so was analysed in a MLR.

Table 6.4: Adjusted[†] final multiple linear regression models for the independent association between PHQ-9 score[‡] at 12-month follow-up and each inflammatory marker[‡] at baseline, after adjusting for baseline PHQ-9 score[‡].

Model	R^2		Inflammatory marker	PHQ-9 Baseline	Age	MVD	SLE	Smoking
Inflammation Score[1]	0.40	B (SE)	0.01 (0.01)	0.58 (0.03)	-0.008 (0.003)	0.19 (0.09)	0.11 (0.06)	0.13 (0.07)
		standardised-b	0.03	0.57	-0.09	0.06	0.06	0.05
		p	0.423	<0.001*	0.005*	0.045*	0.045	0.072
CRP	0.38	B (SE)	0.04 (0.03)	0.56 (0.03)	-0.006 (0.002)	0.20 (0.08)	0.13 (0.05)	0.12 (0.06)
		standardised-b	0.03	0.57	-0.06	0.06	0.07	0.05
		p	0.265	<0.001*	0.022*	0.015*	0.007*	0.049
VEGF	0.38	B (SE)	0.04 (0.04)	0.58 (0.03)	-0.006 (0.003)	0.21(0.09)	0.09 (0.05)	0.12 (0.07)
		standardised-b	0.03	0.57	-0.07	0.07	0.04	0.05
		p	0.308	<0.001*	0.022*	0.018*	0.098	0.079
IL-1RA	0.38	B (SE)	-0.02 (0.04)	0.58 (0.03)	-0.006 (0.003)	0.22 (0.09)	0.09 (0.05)	0.12 (0.07)
		standardised-b	-0.01	0.57	-0.07	0.07	0.04	0.05
		p	0.709	<0.001*	0.023*	0.015*	0.103	0.063
MCP-1[#]	0.38	B (SE)	0.08 (0.04)	0.58 (0.03)	-0.006 (0.003)	0.21 (0.09	0.09 (0.05)	0.11 (0.07)
		standardised-b	0.05	0.57	-0.07	0.07	0.05	0.05
		p	0.073	<0.001*	0.016*	0.017*	0.086	0.084
TGL	0.36	B (SE)	0.09 (0.07)	0.55 (0.02)	-0.004 (0.002)	0.18 (0.08)	0.12 (0.05)	0.11 (0.06)
		standardised-b	0.03	0.56	-0.04	0.06	0.06	0.05
		p	0.234	<0.001*	0.102	0.023*	0.013*	0.055
Adiponectin	0.37	B (SE)	0.01 (0.04)	0.56 (0.03)	-0.004 (0.002)	0.20 (0.08)	0.11 (0.05)	0.11 (0.06)
		standardised-b	0.01	0.56	-0.05	0.07	0.06	0.05
		p	0.744	<0.001*	0.080	0.012*	0.020*	0.066

[1] Composite score of the sum of the z-scores of CRP, IL-1RA, WBC, MCP-1, VEGF and TGL at baseline based on factor analysis (Chapter 4).

[*]significant after Simes' improved Bonferroni correction for multiple testing

[†]adjusted for age, gender, ethnicity, HbA_{1c}, BMI, smoking, history of macrovascular disease (MVD) and prescribed medications (baseline), stressful life events between baseline and 12 months and PHQ-9 score at baseline.

Only PHQ-9 at baseline, age, MVD and Stressful Life Events (SLE) are displayed here as these were the explanatory covariates.

[‡]these variables were ln transformed

[#]MCP-1 was trending close to significance in the unadjusted Spearman's correlation and so was analysed in a MLR.

Table 6.5: Incidence of PHQ-9 depression cases at 12-month follow-up (in participants who were non-depressed subjects at baseline, n = 789) stratified by baseline Systemic Inflammation Score

Variable	Total (n = 789)	Low inflammation Score (n = 402)	High inflammation Score (n = 387)	p-value
Baseline mean age, years	57.6 (11.0)	56.8 (11.4)	58.4 (10.6)	0.043*
Gender (%)				
Male	453 (57.4)	220 (54.7)	233 (60.2)	0.120
Female	326 (42.6)	182 (45.3)	154 (39.8)	
Ethnicity (%)				
White	438 (55.5)	152 (37.8)	286 (73.9)	<0.001*
Black	274 (34.7)	218 (54.2)	56 (14.5)	
Asian/Other	77 (9.8)	32 (8.0)	45 (11.6)	
HbA_{1c}				
Baseline				
Mean % HbA_{1c}	6.92 (1.37)	6.84 (1.34)	7.00 (1.40)	0.081
Mean HbA_{1c},mmol/mol	52.1 (15.0)	51.2 (14.6)	53.1 (15.4)	
12-month				
Mean % HbA_{1c}	6.83 (1.17)	6.77 (1.14)	6.90 (1.19)	0.142
Mean HbA_{1c},mmol/mol	51.2 (12.8)	50.5 (12.5)	51.9 (13.0)	
Body Mass Index				
Baseline				
Mean BMI, kg/m^2	31.5 (6.15)	30.1 (5.21)	32.9 (6.69)	<0.001*
12-month				
Mean BMI, kg/m^2	31.6 (6.04)	30.3 (5.38)	32.9 (6.38)	<0.001*
Baseline macrovascular disease (%)				
None	716 (91.9)	380 (95.5)	336 (88.2)	<0.001*
More than 1	63 (8.1)	18 (4.5)	45 (11.8)	
Baseline-12-month stressful life events				
None	504 (65.7)	263 (67.3)	241 (64.1)	0.356
More than 1	263 (34.3)	128 (32.7)	135 (35.9)	
12-month psychological assessment				
Incident Depression Cases (%)	47 (6.0)	21 (5.2)	26 (6.7)	0.375

***significant α=0.05. T-test and Mann Witney U test used for normal and non-normal data**
Missing or incomplete values for: HbA_{1c} (12 month) = 7 (29) cases, BMI (12 month) = 1 (5) cases, macrovascular history = 10 cases, stressful life events = 22 cases.
Low inflammation scores were scores that were below the mean score at baseline and high inflammation scores were those that were above the mean score at baseline

Discussion

Twelve months after baseline, there was an unadjusted 29% increased incidence of PHQ-9 depression cases in those with increased inflammation compared to those with lower systemic inflammation that was not statistically significant. There was also a statistically significant association between three markers of inflammation as well as the composite marker of inflammation at baseline and PHQ-9 depressive symptom score at 12-month follow-up. After adjusting for relevant confounding variables, including baseline characteristics (adiposity, age, gender, ethnicity, smoking, HbA_{1c}, macrovascular disease and medications with anti-inflammatory action) and stressful life events between baseline and the 12-month follow-up the association remained for CRP, MCP-1 and TGL as well as inflammatory marker score.

A strength of the analyses in this chapter is that there were two data points. This allows some longitudinal associations (involving inflammation and depression in type 2 diabetes mellitus) to be described for the first time. A further strength is that the main outcome variable, the PHQ-9, was used both as continuous variable and as a binary variable with a cut-off of ≥ 10 to indicate probable cases of depression. Use of a binary score allowed for comparisons of the incidence of PHQ-9 depression cases, while the continuous score 0-27 allowed for associations to be measured with increased power while adjusting for confounding variables. Stressful life events and psychosocial stress are associated with an increase in systemic inflammation (Bierhaus et al., 2004) and are a known risk factor for depression (Kendler et al., 1999), so a measure of stressful life events was included in the multiple linear regressions and was a significant confounder. As this was a newly diagnosed type 2 diabetes mellitus population there should not have been any incidence of microvascular diabetes complications (foot ulcers, amputations, advanced retinopathy), which are significantly associated with inflammation (Nguyen et al., 2012) and depression (Pouwer et al., 2003), and could have been a significant source of confounding.

The main limitation of these analyses is that depression cases were determined using the PHQ-9 cut-off score, and a diagnosis of depression was not confirmed using a clinical interview. As the PHQ-9 cut-off of ≥ 10 has a lower specificity than sensitivity, (Twist et al., 2013) there may have been some participants classified as depression cases that were not suffering from major depression. Antidepressants and psychotherapy were not recorded at baseline, and so there may be some participants who were receiving treatment for depression and were not symptomatic at baseline were not excluded from the primary analysis (table 1). Additionally, some cases of depression that occurred after baseline may have been missed if medication had significantly alleviated depressive symptoms before follow-up appointment. As there is no systematic evidence from randomised control trials or observation studies to support a direct pro- or anti-inflammatory effect of antidepressants or psychotherapy, the inclusion of these people as having low PHQ-9 scores may have added a source of error. There is a further risk of residual confounding for poor adherence to medication, which it was not possible to measure, although medication data was derived from current GP prescription records. It was not possible to measure possible causes of the increased inflammation, apart from stressful life events. Some chronic inflammatory conditions may have been included; however terminal and advanced conditions were exclusion criteria so the more severely affected cases were excluded. Oestrogen replacement therapy and any potential inflammatory effects of pre-menopause were not adjusted for but the majority of females in the study population were post-menopausal. Diet and physical activity were not included because self-report measures are not sufficiently accurate, but BMI was used and may be considered a proxy marker for both. As two data points were used and all inflammatory markers were not measured at the second data point, it was assumed that systemic inflammation remained consistent. There is a small risk that comorbid acute inflammatory conditions may have been included in the cohort. Inclusion of those with acute conditions in the group with greater inflammation may have led to an underestimation of the effects of chronic systemically raised inflammation.

In type 2 diabetes mellitus, there is a significantly increased incidence of depression of between 15-24% (Mezuk et al., 2008; Nouwen et al., 2010), although this risk is lower than the increased risk of type 2 diabetes mellitus in depression. From cross-sectional studies it is known that depression in diabetes is associated with worse diabetes complications (Pouwer et al., 2003), and a large retrospective study (over 80,000 subjects) reported no differences in the incidence of depression in newly diagnosed type 2 diabetes mellitus when compared to control (Brown et al., 2006) it may be possible that the psychological and biological stress of late diabetes complications are responsible for this increased incidence. Additionally, inflammation may be a risk factor for earlier diabetes complications, which may be a mediator for inflammation as a risk factor for depression. An alternative hypothesis is increased systemic inflammation is involved in the pathogenesis, and is the common antecedent, of the increased incidence of depression and depressive symptoms and also the earlier diabetes complications. In this chapter the incidence of depression and the depressive symptoms has been studied in a newly diagnosed type 2 diabetes mellitus population that have limited diabetes complications. There was an increased incidence of PHQ-9 depression cases in those with increased inflammation at baseline, which was not statistically significant. There was also an association between inflammation at baseline and depressive symptoms 12 months later. This adds weight to the hypothesis that some of the increased incidence of depression in type 2 diabetes mellitus may be due to increased inflammation.

There are several possible causes for the increased inflammation at baseline, which is associated with depressive symptom score 12 months later. It is possible that the association between inflammation at baseline and depressive symptoms 12 months later is due to the presence of other chronic comorbid conditions with an inflammatory pathogenesis such as rheumatoid arthritis, which is associated with an increased risk of depression in (Brown et al., 2006).

It is also possible that the basal levels of inflammation differ between individuals due to genetic factors. Alternatively, the increased inflammation could have been caused by lifestyle differences (for example reduced exercise or dietary differences) (Pickup, 2004). These are all long-term causes that would mean inflammation levels would be likely to be constant over a period of years and would be unlikely to change without any medical intervention (for example anti-inflammatory medications or dietary changes).

To conclude, irrespective of the causes for the increased inflammation, the associations described partially support the hypothesis that increased systemic inflammation may be a risk factor for the incidence of depression or depressive symptoms reported in type 2 diabetes mellitus, however at this early stage in the course of type 2 diabetes mellitus it is less so than depression is a risk factor for inflammation. Future long-term follow-up of these participants at 24 months, and beyond, may reveal further differences in depressive symptom score and potentially statistically significant differences in the incidence of depression cases between those with more and less inflammation at baseline. Future follow-up with repeated measures will allow for increased power using data imputation. Future research is also required to investigate the main contributors to the raised systemic inflammation observed in type 2 diabetes mellitus to aid the development of specifically targeted future treatments for type 2 diabetes mellitus and comorbid depression.

The next chapter will discuss the key findings from each chapter and the conclusions drawn. Finally, the clinical implications and the implications for future research are discussed.

Chapter 7: Discussion

Synopsis

In this concluding chapter the results of the studies testing the four main hypotheses that were tested are summarised. The strengths and weaknesses of a longitudinal prospective observational cohort study design (with two time points) are discussed. The first main finding was that depressive symptoms at baseline are associated with increased systemic inflammation. The second was that an index of inflammation accounted for more of the variance in depression symptoms than any individual marker. The third was that those with more depressive symptoms at baseline had increased systemic inflammation 12 months later. The final main finding was that inflammation at baseline was not associated with an increase in depressive symptoms 12 months later. The overall conclusion was that those with depression at time of diagnosis with type 2 diabetes have significantly greater concentrations of circulating inflammatory markers and that this increased inflammation may help to explain some of the increased risk of cardiovascular disease and worse type 2 diabetes mellitus prognosis observed in this group (when compared to those without depression). Alternative explanations for the poorer prognosis observed in those with type 2 diabetes mellitus and depression are discussed; these include behavioural aspects of depression such as impaired self-care and sedentary behaviour. The main research implication is that further long-term follow-up of this population is required to further investigate the relationship between inflammation and depression in the context of increased duration of diabetes, including the effects of potential mediating factors such as diabetes complications.

Summary of Main Findings

This thesis studied the cross-sectional and longitudinal relationship between depressive symptoms and inflammation, in a prospective cohort designed to examine the role of psychological factors on type 2 diabetes mellitus. The sample population consisted of 1790 newly diagnosed type 2 diabetes mellitus patients, recruited from 96 primary care practices in South London. This group of patients were selected as this represents a window of opportunity to identify those patients at high risk of poor prognosis with the potential of early interventions. Additionally, those with newly diagnosed type 2 diabetes mellitus are less affected by some potential sources of confounding in the relationship between inflammation, diabetes and depression such as insulin treatment, and late diabetes complications. The study used a prospective population cohort design with a follow-up 12 months after baseline appointment to minimise selection bias.

At baseline the cohort population had a mean age of 56 years, over half were male, almost half were of a Black African/Caribbean ethnic background, two fifths were of a White ethnic background and the remainder were of an Asian/other ethnic background. In other words, this was a representative sample of the multi-ethnic south London population. The majority of this sample had good glycaemic control. The depressive symptom score data was positively skewed with the majority of participants scoring zero. Nevertheless, the prevalence of PHQ-9 depression was 14.6% using a cut-off score ≥ 10 and 11.2% when using a cut-off score ≥ 12.

Cross-sectional Association between Depressive Symptoms and Inflammation

The first aim of this thesis was to investigate whether there was a cross-sectional association between depression or depressive symptoms and inflammation in newly diagnosed type 2 diabetes mellitus. As a result of missing data, analysis of the association between depressive symptoms and cytokines were conducted on a just over two thirds of the cohort. The main finding was that after adjusting for significant covariates (age, gender, ethnicity, BMI, smoking status, history of macrovascular disease, prescription of medications with a recognised anti-inflammatory effect), depressive symptoms were positively correlated with five markers of systemic inflammation (CRP, IL-1RA, MCP-1, TGL, WBC) and not correlated with six markers of inflammation (IL-4, IL-6, IL-10, TNF-α, IL-1β, adiponectin). When comparing unadjusted between groups differences those with depression had 20% higher concentrations of CRP, a circulating inflammatory marker, than those with no depression.

Exploratory Factor Analysis

Having identified that several inflammatory markers are associated with depressive symptoms score, the second aim of this thesis was to identify whether there were any latent factors (consisting of several inflammatory markers) that could be used to create composite measures of inflammation and, if so, whether a composite measure of several markers may explain more of the variance in depressive symptom scores than any individual marker. As a result of missing data, this analysis was conducted on just under two thirds of the cohort. No previous exploratory factor analysis has been done to identify clusters of inflammatory markers associated with PHQ-scores or other depression scales. There is not a detailed enough understanding of the processes of the innate and adaptive immune systems, or of any potential aetiological effects of inflammation in depression, to be able to predict which inflammatory markers were likely to group together. Exploratory factor analysis was the chosen method for

determining the factor structure of 11 inflammatory markers and nine individual PHQ-9 score symptoms. The factor structure was then used to create composite scores of clustered inflammatory markers. The first finding was that the PHQ-9 score split into two factors. The first consisted of 'cognitive' depressive symptoms (which are not similar to common symptoms of type 2 diabetes mellitus). The second was composed of 'whole body' depressive symptoms that may be similar to some symptoms of type 2 diabetes mellitus (such as fatigue and disturbed sleep). The inflammatory markers segregated into three distinct factors which were named; "cytokine 1" (IL-6, IL-1β, TNF-α), which accounted for 23.0% of the variance of the original variables; "cytokine 2" (CRP, IL-1RA, WBC, MCP-1, VEGF, TGL), which accounted for 9.5% of the variance of the original variables; and "cytokine 3" (IL-4, IL-10, IL-1β), which accounted for 5.5% of the variance of the original variables. When composite scores of markers from these three factors were compared with the overall PHQ-9 score, one of the three composite inflammatory scores (cytokine 2) explained more of the variance of PHQ-9 scores (standardised-b = 0.17), than any individual inflammatory marker (standardised-b = 0.13). As it explains more of the variance in depressive symptom score than any individual marker, this composite measure of inflammation is a useful inflammatory construct for cross-sectional comparisons. It may be a useful measure when evaluating longitudinal associations between depressive symptom score and systemic inflammation.

Risk of Increased Inflammation: Prospective Analysis

Having identified a cross-sectional association between concentrations of inflammatory markers, inflammation and depressive symptoms at baseline, the third aim of this thesis was to investigate whether increased depressive symptoms at baseline were associated with increased concentrations of the inflammatory markers CRP and TGL, 12 months later. A subsidiary aim was to determine whether a change in depressive symptoms is associated with a change in the concentrations of circulating inflammatory markers. As a result of missing

data, including that from subjects lost to follow-up, this analysis was conducted on 58% of the sample population.

The main finding was that, as a continuous measure, depressive symptoms at baseline were associated with concentrations of CRP at follow-up after adjusting for covariates including baseline CRP. However, this association was attenuated to a trend after Simes' improved Bonferroni adjustment. When the PHQ-9 was compared using a threshold (to distinguish between depression and non-depressed subjects) improvement in depressive symptoms to a below threshold level was not associated with significant improvement in concentrations of CRP compared to those that remained depression cases. Those that became depression cases after 12 months (who were not at baseline) had significantly higher CRP at baseline and at 12-month follow-up than those that were never depression cases.

Those that were PHQ-9 depression cases at either baseline or 12-month follow-up all had a median CRP (and an adjusted exponentiated marginal mean of lnCRP) that was greater than 3.0 mg/L (above the threshold for high risk of cardiovascular disease). Comparatively the majority of those that were non-depressed subjects at baseline or 12-month follow-up had a CRP that was below 3.0 mg/L at both baseline and 12-month follow-up and so were at significantly lower risk of cardiovascular disease. This physiological difference between those that are depressed at some point in the 12 months versus those that are not appears to be consistent. In other words, although there are some increases in inflammation in response to increase in depression case status, depression appears to be associated with increased concentrations in systemic inflammation that do not change in response to change in depression status.

Risk of Depression: Prospective Analysis

The final aim of this thesis was to investigate whether there was a reverse association i.e. increased inflammation at baseline was associated an increase in depressive symptoms at follow-up. As a result of missing data, including that from subjects lost to follow-up, this analysis was conducted on just over three quarters of the sample population. The main finding was that inflammation at baseline was positively associated with depression symptom score at follow-up; however, this association disappeared when adjusting for baseline depressive symptom score. When the PHQ-9 was compared using a threshold (to distinguish between depression and non-depressed subjects) there was a non-significant 29% difference in the incidence of PHQ-9 depression cases between those that had above average concentrations of CRP at baseline versus those that had below average concentrations of CRP at baseline.

In summary, there was a cross-sectional association between concentrations of circulating markers of inflammation and symptoms of depression shortly after diagnosis with type 2 diabetes mellitus that remained after 12-months. Depressive symptoms were associated with increased systemic inflammation at 12-month follow-up but there were no statistically significant differences in the opposite direction.

Methodological Issues

Strengths

The strengths of this study are that participants were recruited to large prospective cohort study, which aims to reduce the effects of selection bias. A prospective study also allows for the measurement of many explanatory and

outcome variables simultaneously, which it would not have been possible to measure or collect retrospectively. This facilitated the collection of detailed psychological and biological data. The sample population consists of a random sample of newly diagnosed type 2 diabetes mellitus patients from a diverse range of social and ethnic backgrounds, therefore the associations described here are potentially applicable to the global type 2 diabetes mellitus population. This is in contrast to other cohort studies that have investigated depression and inflammation in type 2 diabetes mellitus which were cross-sectional and included predominantly individuals of an Asian or White-European ethnic origin respectively (Labad et al., 2012; Hayashino et al., 2014). The PHQ-9 is a widely used questionnaire in both clinical and research settings and has been validated for use in primary care and diabetes populations (Spitzer et al., 1999; Twist et al., 2013), this makes the research translatable and easy to replicate.

Limitations

The weaknesses of this study are that attrition bias may have influenced the results. Other weakness were: reliance on self-report questionnaire data for depressive symptoms, which is less accurate than using a clinical-diagnosis or semi structured interview tools; the limitations of a large cohort study, in which it is not possible to measure all potential sources of residual confounding. Additionally, this study was community based and blood samples were obtained at varying distances from the lab. As a result, the time from venepuncture to sample processing was often in excess of several hours, and some samples could not be analysed, this reduced power due to missing data and added error to the measurement of some blood markers. A further limitation of observational studies where multiple testing to adjust for covariates is necessary is the increased potential for type 1 errors. In order to balance the additional risk of these errors, the alpha level for multiple comparisons of inflammatory marker differences between groups and association between inflammatory marker concentrations and PHQ-9 score were Bonferroni adjusted using Simes' step-up procedure. Finally, there is a risk of residual confounding from confounders that

were not known at the time, such as antidepressants or that it was not feasible to measure, such as exercise.

Validity of Findings

To interpret these findings it is necessary to discuss the study methods, in particular any biases, residual confounders or other limitations. These must all be taken into account when interpreting the findings.

Selection Bias

Although the cohort study was a prospective design that aims to reduce the effects of selection bias on the sample selection, there is still the potential for selection bias to have had effects on the study population. Three quarters of those eligible responded to the study invitations at baseline. This is considered acceptable for a prospective cohort study. Non-response may have been associated with a systemic bias and led to an over or under-estimation of the prevalence of depression in type 2 diabetes mellitus and of association between depression and inflammation. It is likely that the most severely depressed participants would also have been more likely to have severe anxiety symptoms and may have been less likely to respond to the study, although there is no evidence from the literature to support or disprove this theory. Depressed participants in general may have been more interested in psychological nature of the study and thus it is likely they would have a higher response rate than non-depressed subjects (Ronmark et al., 1999; Kotaniemi et al., 2001). It is likely that selection bias has resulted in an over-representation of those with mild or moderate depression and an under-representation of those with severe depression. In addition, the recurrent nature of depression means that a higher proportion of the sample that were non-depressed at baseline may have had a previous depressive episode. These previous depressive episodes and their risk

factors (such as childhood adversities) may have had a long-term effect on HPA axis function or inflammation.

It was not possible to identify whether non-responders were more or less likely to be depressed, however the cohort recruited well from ethnic minority groups and males, groups which are typically difficult to recruit to long-term studies. Furthermore, the prevalence of depression in the study population is similar to the prevalence of depression reported in other diabetes cohorts (Anderson et al., 2001).

Although the cohort study was a prospective design that aims to reduce selection bias, there is still the potential for attrition bias to have had an effect on those who were included for follow-up analyses. It was not possible to record PHQ-9 scores for non-responders at 12 months but it is possible that participants with more severe depressive or physical symptoms at follow-up were more likely to be lost to follow-up. If this was the case there may have been an attrition bias at follow-up for participants that had less depressive symptoms to remain in the study. This potential bias could have significantly influenced the results and have led to an underestimation of the associations with inflammation and depressive symptoms. However, it is unlikely to have had a major effect, as the majority of those with depression at baseline would still have had depression at follow-up and those with depression at baseline were not more likely to be non-responders at follow-up.

Detection Bias

An inclusion criterion for the study was a recent (< 6 month diagnosis with type 2 diabetes mellitus) and late diabetes complications at diagnosis were exclusion criteria. Therefore, the sample of patients included in the study may have been affected by detection bias. Detection bias may have occurred as patients who are

overweight, have high blood pressure, macrovascular disease, depression, are from high-risk Black and Asian ethnic groups or attend their GP surgery more frequently are more likely to have a blood test that identifies type 2 diabetes mellitus (due to a selective screening for diabetes of in the UK for those considered at high risk of diabetes – NICE guidelines PH38) (NICE, 2012). These patients may be more likely to be diagnosed with type 2 diabetes mellitus at an earlier stage of the condition and therefore would be less likely to have late diabetes complications at diagnosis and be excluded from the study. This may have led to a bias in this sample of newly diagnosed patients for those who were more overweight, had higher blood pressures or were more likely to attend their GP surgery. Patients with non-obesity related type 2 diabetes mellitus and patients with no noticeable diabetes symptoms at diagnosis might, in general, have a different pathogenesis of the condition, which may not be apparent in this sample.

Spectrum Bias

The study population are from a diverse range of cultural and ethnic backgrounds and therefore represent the global type 2 diabetes mellitus population. This means there is the risk of spectrum bias due to cultural differences influencing the results (Cole et al., 1998). The PHQ-9 score for example was designed for use in screening for depression and was tested in western urban population (Spitzer et al., 1999). There is evidence that PHQ-9 scores for those with clinically diagnosed depression may differ for individuals from different cultural and ethnic backgrounds (Huang et al., 2006; Baas et al., 2011). If this is the case in this population it could have led to spectrum bias and those from different cultural and ethnic backgrounds may score differently on the PHQ-9 score, as it may not be measurement invariant. This bias may partly explain ethnic differences in PHQ-9 score and depression observed in this thesis.

Reporting Bias

Gender is a potential source of reporting bias. There are recognised differences in the reporting of depressive symptoms between females and males (Möller-Leimkühler, 2002), and stigma may have a large impact on the reporting of mental health problems (Barney et al., 2006). Such reporting biases are likely at least partly responsible for the differences in PHQ-9 scores between male and female subjects and also for the higher proportion of female subjects in the depressed group.

Hawthorne Effect

A standardised questionnaire was used to ensure that the participants were all asked the questions in the same way. This consisted of a combination of generic self-report tools with established cut-offs indicative of psychological conditions (such as the PHQ-9 for depression) and study specific questions (such as the list of stressful life events). The majority of patients were screened for depression by a healthcare professional shortly before entry into the study. This in combination with the fact that the patients were aware they were taking part in a study, investigating the effect of psychological factors in type 2 diabetes mellitus, may have led to a 'Hawthorne Effect' – where participants change their behaviour or responses as a result of knowing they are being observed while taking part in a study. It is possible that this may have influenced the answers participants gave to questions, their behaviour, or their adherence to medication (McCambridge et al., 2014).

Residual Confounding (Omitted Variable Bias)

A cohort design was chosen for this study and this allowed for the inclusion of measurement and adjustment for a range of potential variables, which could have been potential confounders. Nevertheless, there were variables that it was

not possible to measure or that were not included in the model, omission of these variables could have led to omitted-variable bias.

As the study population were part of a cohort study with a planned follow-up it was not feasible to collect objective data on dietary intake or physical activity; these would have incurred significant extra costs and patient attrition. BMI may be considered a proxy marker of both long-term diet and physical activity, however there are acute effects of dietary differences and exercise on inflammation and depressive symptoms which would not necessarily correlate with BMI (Ploeger et al., 2009). It is possible that exclusion of this variable may have led to a small underestimation of the independent relationship between inflammation and depressive symptom score.

As with all epidemiological studies it was not possible to measure, and therefore adjust for, all potential sources of confounding. Variables that were not measured that may have been confounders, and could have led to this bias, include: diet, the acute and long-term effects of exercise, antidepressant medications, psychotherapy, acute and chronic comorbid conditions that were not exclusion criteria for the study, such as rheumatoid arthritis or Crohn's disease.

There is evidence that certain diets can lead to acute increased circulating FFAs (free fatty acids) which have pro-inflammatory effects (Wilding, 2007) and depression and anxiety are associated with poor diet (Bonnet et al., 2005). There are measurable acute effects of exercise on increasing inflammation (Ploeger, Takken et al. 2009) and long-term anti-inflammatory effects (Ford 2002, Kadoglou, Perrea et al. 2007). It was recognised that the long-term effects of exercise may have been an important confounder as depression is associated with more sedentary behaviour and so BMI, which may be considered a proxy marker of diet and exercise, was adjusted for. Nevertheless, the acute effects of

diet and exercise may play an important role in the association between inflammation and depression in this type 2 diabetes population.

There are not many detailed studies with a sound methodology that demonstrate antidepressants have direct pro-inflammatory or anti-inflammatory effects. Meta-analyses have demonstrated some antidepressant medications are associated with increased weight gain, and possible pro-inflammatory effects (Serretti and Mandelli, 2010). Some case control studies and observational studies of small numbers of patients have also reported anti-inflammatory effects (Lanquillon et al., 2000) and effects on glucose regulation (Khoza and Barner, 2011) and similar effects have been reported in meta-analyses (McIntyre et al., 2006); however the mode of action for these effects has not yet been established. It has been proposed that there are direct pro-and anti-inflammatory effects of these antidepressants but as the associations reported are small and often become non-significant after adjusting for confounders (Barnard et al., 2013). As such there is a risk that residual confounding due to behavioural changes, which may occur as a result of the reduction in depressive symptoms, or other diabetes risk factors that have not been adjusted for may be the cause of these associations. If there are some small pro-or anti-inflammatory effects of antidepressant medication that are independent of weight gain this may have been a significant source of confounding. Some antidepressant use may have caused an underestimation in the relationship between depressive symptoms and inflammation while other antidepressant use may have caused the opposite. Overall the effects of antidepressants are complex and poorly understood.

Acute and chronic conditions such as upper-respiratory tract infections, rheumatoid arthritis and cancer are associated with an increase in systemic inflammation (Coussens and Werb 2002). For example, CRP is typically between 10-40mg/L during upper respiratory tract infections and between 40-200mg/L for bacterial infections (Clyne and Olshaker, 1999). These conditions are also

associated with fatigue and sleeping abnormalities, which can be very similar to some of the symptoms of depression when it is assessed by the PHQ-9 (Krause et al., 2008; Richardson and Richards, 2008). Acute conditions would only have significantly confounded the cross-sectional analyses however chronic long-term conditions such as rheumatoid arthritis or Crohn's disease may have been significant sources of confounding in the long-term analyses.

Some other factors that were not measured were chronic stressors but not sudden life events, such as domestic and workplace stress or financial difficulties. Although physical stress and homelessness were recorded, less severe financial difficulties such as debt, having less money to pay bills, and less severe workplace or domestic problems such as verbal abuse or bullying were not. At the time of data collection schedule development these were not considered, and stressful life events were prioritised as it is not feasible to measure all sources of potential confounding and measures for life events are perhaps less prone to reporting bias than measures for chronic stressors.

In addition, there may be other unknown factors that have yet to be identified that may have confounded the results. Although it is unlikely that there are other major or strongly confounded residual factors that could have significantly confounded or biased the results, the evidence base for new variables that should be considered is constantly developing. This is a limitation that must be considered when interpreting the results.

There were most probably some bias effects on the study, however it is unlikely that these account for all of the difference in concentrations of CRP between those who were depression cases and those that were not. Furthermore, it is unlikely that bias completely accounts for the cross-sectional associations in this study between markers of inflammation and depressive symptoms.

Power

Power is the probability of a test correcting identifying a statistically significant difference when such a difference actually exists or, more simply, avoiding a type 2 error. Negative cross-sectional and longitudinal findings are unlikely to be due to chance as they were observed in representative samples with sufficiently large sample sizes, additionally cross-sectional findings are in keeping with numerous other studies (Labad et al., 2012; Hayashino et al., 2014).

This study had a large sample size that exceeded the conventional levels of power (80%) to detect a difference in CRP between depressed and non-depressed subjects at the 5% significance level, thus minimising the risk of type 2 errors. As large sample sizes were used for adequate power there was a risk of observing statistically significant differences, which were not clinically significant. Without knowledge of what constitute clinically significant differences in the concentrations of specific biomarkers or questionnaire measures it is not possible to understand whether differences are clinically relevant. For example, the median concentrations of CRP are statistically different between those that were depression cases at baseline and those that became depression cases 12 months later; however the concentrations of CRP for both groups were greater than 3 mg/L (the threshold for high risk of cardiovascular disease when using this measure). Therefore, the differences in systemic inflammation between these groups may not be clinically relevant. Conversely the median concentrations of CRP are statistically different between the depressed and non-depressed groups. However, the differences in systemic inflammation mean that the majority of each respective group are in different categories for risk of cardiovascular disease, therefore this difference is both clinically and statistically significant. The large sample size also allowed for more complex statistical techniques to be applied such as multiple linear regression, exploratory factor analyses and ANCOVA.

Despite having a large sample size of newly diagnosed type 2 diabetes patients (n = 1790) there was only a relatively small sample of depressed individuals (n = 220), therefore between-group comparisons of depression vs. non-depressed subjects were underpowered. As there was a very small sample of 'incident depression' (n = 47), and the odds ratio between groups with high and low inflammation was approximately half of that which was expected after 12 months, there was insufficient power to identify differences in incident PHQ-9 depression. A retrospective power analysis confirmed that 3904 participants per group would be required after 12 months and 1828 per group would be required after 24 months for 80% power at the 5% significance level. However this is the largest new onset cohort of diabetes in the UK and it would be necessary to recruit even larger samples to generate a higher absolute numbers of depressed patients, adding to cost, time and resources.

A face-to-face interview with use of self-report questionnaires was used to determine depressive symptoms and to collect data on a range of potential confounders such as stressful life events. Use of large cohort study for an increased sample size and more statistical power was at the expense of the use of self-report measures – because semi-structured clinical interviews/diagnoses were too time-consuming and costly. Self-report measures, such as the PHQ-9, are not as accurate as clinician-based measures using semi-structured interviews such as the SCAN 2.1. This is a common limitation of the assessment of psychiatric disorders in epidemiological studies. However, to confirm that the data was accurate, self-report data for depression was validated with a semi-structured tool for clinical diagnosis (SCAN 2.1). In this newly diagnosed type 2 diabetes mellitus population the PHQ-9 cut-off of ≥ 10 has a lower specificity for depression (70 %) than has been reported when used in the general population (98 %) (Spitzer et al., 1999; Twist et al., 2013). A cut-off of ≥ 12 gives the ideal specificity in this population (80 %), although at the cost of a reduced sensitivity of 87 % (compared to a sensitivity of 95% at the PHQ-9 cut-off of ≥ 10) (Twist et al., 2013). One advantage of using self-report measures such as the PHQ-9 for this thesis is that they are a continuous measure of depressive symptoms. This

gives the possibility to analyse the variance of depressive symptoms whereas a binary outcome such as a clinical diagnosis does not, further increasing the power of statistical tests.

Interpretation

Depressive symptoms at baseline were associated with CRP 12 months later, even after adjusting for baseline concentrations of CRP. Furthermore, there was a significant increase in CRP in those that became 'new' PHQ-9 depression cases after 12 months compared to those that did not. This suggests that those with type 2 diabetes mellitus and comorbid depression at baseline have persistently raised concentrations of circulating inflammatory markers that do not decrease with the abatement of depressive symptoms and diverge from the concentrations in the non-depressed type 2 diabetes mellitus population.

Those with more depressive symptoms have increased concentrations of circulating inflammatory markers (CRP, IL-1RA, MCP-1, WBC, TGL) than those with less depressive symptoms. During the course of this project similar findings have been published elsewhere, such as the association of leptin, and of CRP, with depression in type 2 diabetes mellitus (Labad et al., 2012; Hayashino et al., 2014). These associations may be truly independent or may have been overestimated due to residual confounding. Furthermore, whether inflammation is the antecedent of the depressive symptoms, or vice versa, cannot be deduced from these cross-sectional associations.

Potential Mechanisms for the Association between Depression and Inflammation

This thesis partly investigated whether inflammation was the antecedent for depression, type 2 diabetes mellitus and cardiovascular disease as there is evidence that suggests inflammation may be a risk factor for both depression and diabetes individually. The association between inflammation and depressive symptoms in type 2 diabetes mellitus is present at diagnosis with type 2 diabetes mellitus, and 1 year later. There are a number of mechanisms that may explain this association of depressive symptoms and inflammation in type 2 diabetes. The first includes physiological processes such as systemic inflammation and HPA axis dysfunction.

Depression in the general population, when defined by diagnostic clinical interview, has a small but significant association with a systemic, cytokine-mediated chronic inflammatory state (a marker of activation of innate immunity) (Simon et al., 2008; Dowlati et al., 2010; Lamers et al., 2013). Insulin resistance, type 2 diabetes mellitus and cardiovascular diseases are also associated with a chronic low-grade inflammatory response due to activated innate immunity (Pickup et al., 1997; Kaptoge et al., 2010; Fernandez-Real and Pickup, 2012). Prospective studies have shown that raised circulating concentrations of inflammatory markers, including acute-phase proteins such as CRP and pro-inflammatory cytokines such as IL-6 are associated with the onset of type 2 diabetes mellitus in cohorts with initially normal glucose tolerance (Thorand et al., 2003; Pickup, 2004). Activated innate immunity is also a strong independent risk factor for cardiovascular events and mortality in the general population and in those with established type 2 diabetes mellitus (Ridker et al., 2002; Pickup and Mattock, 2003; Kaptoge et al., 2010). There have been similar observations of associations between inflammation and cardiovascular disease; in a cohort of people with depression, those with asymptomatic cardiovascular disease had increased concentrations of markers of systemic inflammation (Rajagopalan et al., 2001). Furthermore, childhood adversity, a major risk factor for adult

depression, is prospectively associated with higher concentrations of CRP in early adult life (Hartwell et al., 2013). Depressive symptoms are associated with raised white blood cell count in people with established cardiovascular disease (Duivis et al., 2013). In a small selected inpatient depression sample (n = 70), adiponectin and IL-6 were associated with metabolic status (Zeugmann et al., 2010) and in a secondary analysis of an elderly U.S. cohort, a nested sample of established type 2 diabetes mellitus (n = 14), depressive symptoms were positively associated with IL-6 (Doyle et al., 2013).

As was explored in Chapter 1, there are a number of plausible mechanisms by which the increased concentrations of circulating inflammatory markers observed in this newly diagnosed type 2 diabetes mellitus population may be involved in the pathogenesis of depression. These include reducing 5-HT via the indoleamine-2,3 dioxygenase pathway, reducing 5-HT uptake, altering HPA axis function and altering the permeability of the blood brain barrier. Although the inflammatory markers most closely associated with the function of the HPA axis (TNF-α, IL-6) were not significantly associated with depressive symptoms, this may have been due to the circadian nature of the HPA axis and the fact that time of venepuncture was not controlled for.

Psychological and behavioural effects of depression are an alternative mechanism that may explain the increased inflammation observed in depression. Behavioural aspects of depression may include poorer diet, reduced diabetes self-care, less exercise and smoking, which may be the cause of increased inflammation. While potential confounding was adjusted for, it is never possible to adjust for all sources of potential confounding in epidemiological studies. Consequently the associations described in this thesis may be the result of residual confounding. For example, history of depression, childhood adversity and history of antidepressant medication therapy were not adjusted for. A large proportion of those with increased depressive symptoms in this population may have had recurrent patterns of depression for many years. Altered behaviour

over this time may have led to increased inflammation in these participants (Winkley, 2010). Furthermore, there is recent evidence which suggests that some antidepressant medications may induce hyperglycaemia (Barnard et al., 2013). There are no meta-analyses that show direct anti-inflammatory and pro-inflammatory effect of antidepressants, so antidepressants were not adjusted for in the study. However, it is possible that the antidepressant medication caused some residual confounding.

Thirdly it is possible that some of the depressive symptoms detected (such as fatigue and sleeping abnormalities) were psychological manifestations of increased systemic inflammation and, in some cases, were not actually symptoms of depression. Recent factor analyses of the PHQ-9 in populations with chronic comorbid conditions have revealed a two-factor structure of the PHQ-9 (Krause et al., 2008; Richardson and Richards, 2008). Commonly one factor contains symptoms more commonly associated with the construct of depression (anhedonia, low-mood, guilt, self-harm, self-harm ideations). The other factor contains symptoms that are also associated with the construct of depression but also are common symptoms of the acute phase response, type 2 diabetes and other chronic inflammatory conditions (sleep disturbances, tiredness, altered appetite, reduced concentration) (Krause et al., 2008; Richardson and Richards, 2008). The two-factor structure of the PHQ-9 identified in these studies suggests that PHQ-9 symptoms that overlap with common symptoms of other chronic conditions and this may reduce the specificity of the PHQ-9 cut-off of ≥ 10 when used in populations with co-morbid long-term conditions.

There is also a two-factor structure of the PHQ-9 in this study population and a reduced specificity at a cut-off of ≥ 10 (Twist et al., 2013). This supports the hypothesis that more individuals who are not clinically diagnosed depressed are scoring ≥ 10 on the PHQ-9 in when compared to the general population due to the presence of multiple symptoms (perhaps related to systemic inflammation) that are being reported in the PHQ-9. This increased systemic inflammation may

in turn be due to a longer duration of undiagnosed type 2 diabetes, other comorbid chronic conditions, or genetic and environmental factors previously discussed. It is noted that even when using a higher PHQ-9 cut-off of ≥ 12 the between group differences of inflammatory markers between depression cases vs. non-depressed subjects were generally as large or greater than when using the cut-off of ≥ 10 (Table 3.3a & Table 3.3b), and the factor analyses identified a similar association between both PHQ-9 symptoms which may not be symptoms of depression (factor P2) and those which are (factor P1) with markers of systemic inflammation. Therefore, it appears that these symptoms of increased systemic inflammation do not explain all of the associations described.

Potential Mechanisms for the Increased Incidence of Depression in Type 2 Diabetes Mellitus

As there is an increased incidence of depression in type 2 diabetes mellitus it was hypothesised that some of the depression in type 2 diabetes mellitus may be explained by increased systemic inflammation. Multiple-linear-regression was used to ascertain whether systemic inflammation measured shortly after diagnosis with type 2 diabetes mellitus was associated with depressive symptom score 12 months later after adjusting for relevant potential sources of confounding. In this investigation the association between inflammatory markers at baseline and depressive symptom score at follow-up was attenuated after adjusting for depressive symptom score at follow-up. Therefore, there is no independent association between inflammatory markers at baseline and depressive symptoms 12 months later. This suggests that increased inflammation shortly after diagnosis with type 2 diabetes mellitus is not associated with an increase in depressive symptoms at follow-up. Based on the findings of this thesis, at the early stages of type 2 diabetes mellitus, depression appears to be a more significant risk factor of worsening inflammation than inflammation being a risk factor for depression. This is expected as previous meta-analyses suggest that depression is approximately a 2-fold greater risk factor for diabetes than vice versa.

There are a range of psychiatric comorbidities that commonly occur in depression such as migraine, anxiety and insomnia (Mineka et al., 1998). Additionally, those with depression often develop or are diagnosed with other physical comorbidities, such cardiovascular disease or type 2 diabetes mellitus, at an earlier age (Brown et al., 2005) (Laake et al., 2014). Some of the increased risk for these comorbid conditions (such as type 2 diabetes mellitus or cardiovascular disease) may be in part explained by behavioural characteristics associated with depression, for example, reduced self-care (Ciechanowski et al., 2000), sedentary lifestyle (Teychenne et al., 2010), reduced quality of life (Schram et al., 2009), and less social support (Cohen and Wills, 1985; Holahan and Holahan, 1987). There is an increased incidence of depression in conditions such as type 2 diabetes mellitus of 15-24% (Mezuk et al., 2008; Nouwen et al., 2010). The clustering of conditions may have a physiological or physiological basis, or a combination of the two. The findings of this thesis suggest that concentrations of circulating inflammatory markers at diagnosis with type 2 diabetes are associated with the change in depressive symptoms in the 12-18 months after diagnosis with type 2 diabetes mellitus.

These findings, in conjunction with previous studies that demonstrate that depression in type 2 diabetes mellitus is also associated with diabetes complications and mortality (Winkley et al., 2012), imply that the increased incidence of depression previously observed in type 2 diabetes mellitus may also be related to other factors (as a result of the increased burden of living with and managing a chronic condition with complications such as diabetes foot ulcers).

It is possible that cases of depression that share a common inflammatory physiological basis with type 2 diabetes mellitus either occur before the development of diabetes or much later in the course of condition. There was already a significant association between inflammation and depression at baseline, but it is not possible to determine the direction of this association.

Furthermore, diabetes complications are associated with depression in type 2 diabetes and, as increased inflammation is a risk factor for some diabetes complications, these may be an important mediator in the relationship between inflammation and depression. If this is the case it would explain why no association was apparent so soon after diagnosis with the condition.

Finally, the sample size used only included 47 cases of 'incident depression', and the monitoring period was only for 12 months so there may have been insufficient power to identify clinically significant differences in the incidence of depression. If the relationship between inflammation at baseline and depression at follow-up is non-linear that may explain why no association was evident from the multiple regression.

Implications

There are several key clinical and research implications from the current literature and the findings of this thesis together with evidence from other research studies.

Research Implications

The majority of those who were depression cases at baseline and the 12-month follow-up had concentrations of CRP that were indicative of high risk of cardiovascular disease (> 3 mg/L) (Pfutzner and Forst, 2006). Further research is required to identify potential mechanisms by which CRP may be involved in the pathogenesis of depression in type 2 diabetes mellitus.

In the 12-month period of observation in this study, increased systemic inflammation at baseline was not associated with a significant difference in future PHQ-9 depression cases, nor was it associated with a difference in the change in depressive symptom score from baseline to 12-month follow-up. There was however around 30% increased incidence of depression cases in the group with increased systemic inflammation at baseline. It would be interesting to study this association in future research, with larger sample sizes. More importantly such work should control for history of depression, to further examine if inflammation is a risk factor for depression in type 2 diabetes mellitus. In order to generate the large sample sizes needed, the use of large retrospective cohorts with linked data from electronic healthcare records could be considered. I would hypothesise that inflammation is a risk factor for depression and that later on in the course of type 2 diabetes mellitus a proportion of this risk is mediated by the development of late diabetes complications, due to the association between depression and late diabetes complications and a plausible common inflammatory aetiology. Additionally investigation of a pre-diabetes population from birth measuring a range of exposures and biomarkers over time could be considered to examine whether inflammation is a risk factor for depression before the development of type 2 diabetes mellitus, such as the ALSPAC study – a cohort of 14,000 21-23 year olds who have been monitored since the eighth gestational week (Golding, 1990).

Further long-term follow-up of this cohort is also required to investigate whether endogenous peripheral inflammation is a risk factor for the development of non-cytokine therapy (IFN) induced depression later in the course of type 2 diabetes mellitus, and whether diabetes complications are a mediator for an association between inflammation and future depression. This will slightly expand the sample size as multiple time-points with repeat-measures will allow for imputation of data, but as time goes on the sample size will further decrease due to attrition. This highlights the potential benefits of open diabetes research registers.

If inflammation is involved in the aetiology and pathogenesis of depression it may be possible that some medical interventions that have an anti-inflammatory effect may also have an antidepressant effect by reducing systemic inflammation, and therefore the proportion of depressive agents which cross the blood-brain barrier.

A novel therapeutic way to reduce inflammation is by increasing physical exercise (Ford, 2002; Kadoglou et al., 2007), interestingly increase in exercise has also been associated with a reduction in depressive symptoms and there is evidence that increasing physical exercise results in reduced inflammation and improvement in depressive symptoms (Eyre and Baune, 2012). Future research is required to see whether interventions to increase exercise can have antidepressant and anti-inflammatory effects that improve the prognosis of those with type 2 diabetes mellitus and comorbid depression.

The potential benefits of anti-inflammatory agents in depression have not yet been widely explored, although there is evidence to suggest that they could be beneficial. There are currently four agents known to be associated with a reduction in depressive symptoms, aspirin, celecoxib (Akhondzadeh et al., 2009; Pasco et al., 2010), infliximab and etanercept (Persoons et al., 2005; Tyring et al., 2006). Of these aspirin, has a very limited effect, needing 10 years of treatment to show any significant effects (Pasco et al., 2010) but it is able to improve efficacy of SSRIs in those who were previously non-responsive (Brunello et al., 2006; Mendlewicz et al., 2006). COX-2 inhibitors such as celecoxib have also been shown to have a significant benefit on improving depressive symptoms after a 6-week treatment period when used in combination with antidepressants, but there is no evidence that they are effective at improving depressive symptoms when used alone. Infliximab and etanercept are monoclonal antibodies that inhibit TNF-α, an adipokine that is secreted by macrophages, lymphocytes, Natural Killer (NK) cells and neurons. TNF-α regulates immune cells and can

induce apoptosis and inflammation as part of the acute phase response. It is not clear how the anti-inflammatory actions of these medications could have antidepressant effects. Future research is required to investigate the effects of anti-inflammatory medications and whether they can have antidepressant effects that improve the prognosis of those with type 2 diabetes mellitus and comorbid depression.

Clinical Implications

The increased prevalence of depression in type 2 diabetes mellitus and the association of depression with worse diabetes outcomes has led to the introduction of recent US ADA guidelines "Standards of Medical Care in Diabetes 2013" and UK NICE guidelines alerting diabetes physicians to the need to screen, identify and treat depression at the earliest stages of diabetes (NICE, 2008; ADA, 2013). In Chapter 3 it was noted that even at diagnosis up to 15% of type 2 diabetes mellitus patients had probable clinical depression, 2 to 3 times more than the prevalence in the general population. Furthermore, depression is associated with increased concentrations of systemic inflammation even within a few months after diagnosis with type 2 diabetes mellitus and this association is independent of other confounders including adiposity and previous macrovascular events. Critically this association persists even after improvement of depressive symptoms. These findings reaffirm the need to screen for, diagnose and treat depression early on in the condition and underline the importance of the ADA and NICE guidelines.

Conclusion

In conclusion, increased concentrations of circulating inflammatory markers, in particular CRP, are associated with increased risk of macrovascular disease. The physiological differences between depression cases and non-depressed subjects at baseline demonstrate that there are already clinically significant physiological

differences between the two groups shortly after diagnosis. These differences may explain the increased risk of macrovascular disease and mortality in this group, since inflammation is a known risk factor for cardiovascular disease and mortality in participants with and without diabetes (Ciechanowski et al., 2000; Lustman et al., 2000; Ismail et al., 2007). This may explain why treating depression and depressive symptoms does not always improve diabetes outcomes (de Groot et al., 2001; Katon et al., 2005), as the increased inflammation and accelerated disease pathology may not be resolved when using conventional pharmacological and psychological treatments for depression. This may help clinicians to better understand and detect diabetes in those with depression that do not fit the usual phenotype for diabetes and cardiovascular disease risk.

Improvement of depressive symptoms to a sub-depression threshold was not associated with a significant improvement in inflammatory markers in this population. This finding in conjunction with previous literature, which demonstrates that treating depression does not lead to improvements in diabetes outcomes (Ismail et al., 2007), supports the theory that increased inflammation is responsible for the increased morbidity in those with depression and type 2 diabetes mellitus.

There is conflicting evidence from collaborative care interventions, where both depression and type 2 diabetes are targeted have shown improvement in diabetes outcomes in patients with both conditions (Katon et al., 2010), this suggests that some causes of the worse prognosis may be behavioural or behaviour modifiable.

This thesis has examined the association of a range of markers of inflammation with depressive symptoms in a newly diagnosed type 2 diabetes population. Original contributions to knowledge include a cross-sectional association

between depression and inflammation and also a longitudinal association between depressive symptom score and increased inflammation 12 months later. There is no significant evidence that inflammation is associated with an increased risk for depression or an increase in depressive symptoms in the 12-18 months after diagnosis with type 2 diabetes mellitus. Depression appears to be driving increased inflammation at the beginning of the course of type 2 diabetes mellitus rather than the reverse. Future long-term follow-up of this cohort is needed to determine whether inflammation is a risk factor for depression later on in the course of type 2 diabetes mellitus and to determine the relative risk of inflammation and depression for the premature development of diabetes complications and mortality.

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Appendix

Full Methods for the measurement of HbA1c

Equipment

Percentage HbA1c was measured using Primus Ultra2 boronate affinity HPLC. The Ultra2 is manufactured by Primus Corporation, 4231 E. 7th Terrace, Kansas City, MO 64132. Within the UK it is distributed by Siemens Limited, Glyn Rhonwy, Llanberis, Caernarfon, Gwynedd LL55 4EL.

Specimen Collection and Sample Storage

Whole blood specimens are required for measurement of HbA_{1c}. EDTA and fluoride oxalate are suitable anticoagulants and were used for measurement of HbA_{1c} for this study. Samples were collected in 2ml fluoride oxalate or 3ml EDTA vacutainers or capillary tubes. Samples were analysed on the day of venepuncture or stored at 4°C overnight before analysis. This is within the requirements for clinical measurement of HbA1c (storage at 4°C for up to one week at laboratory).

Method

Glycated haemoglobin is formed by the attachment of a sugar molecule to the binding site of haemoglobin by a ketoamine bond. 1,2–cis-diol groups are formed in this process and, as these 1,2–cis-diol groups are not found in non-glycated haemoglobin, provide the basis for separation of glycated and non-glycated components of haemolysate (lysed red blood cells) by boronate affinity chromatography. In the technique a boronate (in this case aminophenylboronic acid) is bonded to the surface of a column. When haemolysate is passed through the column, the diol groups of glycated haemoglobin complex with the boronate and cause it to remain in the column. The non-glycated component elutes from the column. Finally, the glycated component is eluted from the column with a reagent that displaces it from the boronate.

The Primus Ultra2 system setup consisted of four main components used to fractionate and quantitate HbA1c:

1. The Ultra2 boronate affinity HPLC (composed of a dual high-pressure pump system, column oven and variable wavelength detector).
2. The Gilson 215 auto-sampler is able to handle 285 samples during a single batch run, with the ability to accommodate a variety of sample tubes with corresponding rack types.
3. The system was interfaced to a computer and printer for system control and data storage.

4. Patient primary identification is made through a handheld barcode scanner linked directly to the computer.

The Primus Ultra2 combines the principles of boronate affinity and high performance liquid chromatography. Low and high-pressure pumps transfer reagents through an analytical column. The analytical column contains aminophenylboronic acid bonded to a porous polymer support. The haemolysate is automatically injected onto the column during the flow of reagent A. The glycated haemoglobin binds to the aminophenylboronic acid, while the non-glycated haemoglobin passes through the column to the spectrophotometric detector, where it is detected at a wavelength of 413 ± 2 nm. After complete elution of the non-glycated haemoglobin, the pump switches to reagent B, this displaces the glycated haemoglobin and passes through the detector where it is detected at the same wavelength. To ensure a stable baseline the composition of the two reagents has been designed so that they exhibit virtually identical absorption. The detector signal is also referenced against a photodiode by the split beam technique. Finally, the column is re-equilibrated with reagent A. The chromatogram shows two peaks – peak 1: non-glycated haemoglobin, peak 2: glycated haemoglobin. The percentage glycated haemoglobin result is calculated using the following formula:

Area of peak 2 ÷ (Area of peak 1 + Area of peak 2) x 100

Percentage HbA_{1c} measurement by boronate affinity chromatography are free from interferences such as haemoglobin variants, non-glycation modifications and storage related haemichromes. No sample pre-treatment to remove the liable Schiff / aldimine is required, since only stable (ketoamine-linked) HbA_{1c} is retained by the boronate. Compared to ion exchange techniques, affinity separation is also less sensitive to quantitative errors caused by minor fluctuations in reagent pH and ionic strength (Biotech).

Conversion from DCCT to IFCC units for HbA1c was done using the following formula:

(%HbA1c – 2.15) x 10.929

<u>Quality Controls</u>

Internal quality controls were run with each batch of samples (Biorad Lyphochek Diabetic Control levels 1 and 2). The inter assay variation at each level was < 5%. Assay performance was also monitored retrospectively via participation in the WEQAS external quality assurance scheme.

Full Methods for the measurement of triglyceride

Equipment

Concentration of triglyceride was measured using the Siemens Advia 2400. The Advia 2400 is manufactured by Siemens Healthcare Diagnostics Ltd, Newton House, Sir William Siemens Square, Frimley, Camberley, Surrey, GU16 8QD. Analysis of HDL Cholesterol on the Siemens Advia was conducted using reagents supplied by the manufacturer.

Specimen Collection and Sample storage

Serum or heparinised plasma are required and were used for measurement of triglyceride concentrations. For all samples measured in the study laboratory concentration of triglyceride was measured using serum plasma from 5ml seroseparator vacutainers. For data collected from GP electronic healthcare records heparinised plasma may also have been used for measurement of triglyceride concentration. Specimens were spun on the day of venepuncture and analysed immediately or stored at room temperature in seroseparator tubes and analysed the following day. The ADVIA 2400 only uses 2 µl of sample for the test, but the minimum volume required in the specimen container was 200µl for the seroseparator vacutainers.

Method

The Siemens Advia method for the measurement of triglycerides is a three-step enzymatic assay. Triglycerides are converted to glycerol and free fatty acids by the lipase enzyme. The glycerol is then converted to glycerol-3-phosphate by the glycerol kinase enzyme. The glycerol-3-phosphate is then converted to hydrogen peroxide by the glycerol-3-phosphate-oxidase enzyme. The hydrogen peroxide forms a coloured complex with 4-aminophenazone and 4-chlorophenol under the catalytic influence of the peroxidase enzyme. The absorbance of this complex is measured at a wavelength of 505/694 nm (FDA).

Sensitivity

The minimum detectable concentration of triglyceride was 0.1 mmol/L

Quality Controls

The inter- and intra-assay CV are < 5%. The ADVIA triglyceride method measures total glycerols and is traceable to a reference method, which uses reference materials from the National Institute of Standards and Technology (NIST), via patient sample correlation. Assigned values of Bayer Chemistry Calibrator and Bayer Assayed Chemistry Controls are traceable to this standardization.

Full Methods for the measurement of HDL Cholesterol

Equipment

Concentration of HDL cholesterol was measured using the Siemens Advia 2400. The Advia 2400 is manufactured by Siemens Healthcare Diagnostics Ltd, Newton House, Sir William Siemens Square, Frimley, Camberley, Surrey, GU16 8QD. Analysis of HDL Cholesterol on the Siemens Advia was conducted using reagents supplied by the manufacturer.

Specimen Collection and Sample Storage

Serum or heparinised plasma are required and were used for measurement of triglyceride concentrations. For all samples measured in the study laboratory concentration of HDL cholesterol was measured using serum plasma from 5ml seroseparator vacutainers. For data collected from GP electronic healthcare records heparinised plasma may also have been used for measurement of triglyceride concentration. Specimens were spun on the day of venepuncture and analysed immediately or stored at room temperature in seroseparator tubes and analysed the following day. The ADVIA 2400 only uses 2 µl of sample for the test, but the minimum volume required in the specimen container was 200µl for the seroseparator vacutainers.

Method

The Siemens Advia Direct HDL cholesterol method is a two-step automated procedure. In the first step non-HDL cholesterol is consumed by the cholesterol esterase and cholesterol oxidase enzymes, leaving a colourless end product. Additional hydrogen peroxide produced is removed from the sample by the catalase enzyme. The absence of detergent in this first reaction prevents HDL from reacting with the enzymes. In stage 2 adetergent is added which solubilises HDL cholesterol and allows it to react with the enzyme system. Sodium azide is added to inhibit the reaction of the hydrogen peroxide formed from stage 2 with catalase already present in the sample. The hydrogen peroxide acts with 4-aninoantipyrine to produce a red quinone amine dye measured at a wavelength of 596 nm.

Sensitivity

The minimum detectable concentration of HDL cholesterol was 0.1 mmol/L

Quality Controls

The inter- and intra-assay CV are < 5%. The Advia HDL method is traceable to the NCEP Designated Comparison Method (reference method) via patient sample correlation. Assigned values of ADVIA Chemistry HDL/LDL Cholesterol Calibrator and ADVIA Chemistry Lipid Controls are traceable to this standardization.

Full Methods for the measurement of Total Cholesterol

Equipment

Concentration of total cholesterol was measured using the Siemens Advia 2400. The Advia 2400 is manufactured by Siemens Healthcare Diagnostics Ltd, Newton House, Sir William Siemens Square, Frimley, Camberley, Surrey, GU16 8QD. Analysis of HDL Cholesterol on the Siemens Advia was conducted using reagents supplied by the manufacturer.

Specimen Collection and Sample Storage

Serum or heparinised plasma are required and were used for measurement of total cholesterol concentrations. For all samples measured in the study laboratory concentration of HDL cholesterol was measured using serum plasma from 5ml seroseparator vacutainers. For data collected from GP electronic healthcare records heparinised plasma may also have been used for measurement of triglyceride concentration. Specimens were spun on the day of venepuncture and analysed immediately or stored at room temperature in seroseparator tubes and analysed the following day. The ADVIA 2400 only uses 2 µl of sample for the test, but the minimum volume required in the specimen container was 200µl for the seroseparator vacutainers.

Method

Serum cholesterol is determined using a single step enzymatic method. Cholesterol esterase completely hydrolyses cholesterol esters in serum to free cholesterol, which is in turn oxidised by cholesterol oxidase generating hydrogen peroxide. The hydrogen peroxide formed combines with 4-aminophenazone and a phenol to form a red quinone amine dye which is measured at a wavelength of 505/694 nm.

Sensitivity

The minimum detectable concentration of HDL cholesterol was 0.1 mmol/L

Quality Controls

The inter- and intra-assay CV are < 5%. The ADVIA cholesterol method is traceable to the CDC reference method, which uses reference materials from the National Institute of Standards and Technology (NIST), via patient sample correlation.

Full Methods for the measurement of LDL Cholesterol

LDL cholesterol was measured using the Freidewald formula.

Full Methods for the measurement of CRP

Equipment

Concentration of CRP was measured using the Siemens Advia 2400. The Advia 2400 is manufactured by Siemens Healthcare Diagnostics Ltd, Newton House, Sir William Siemens Square, Frimley, Camberley, Surrey, GU16 8QD. Reagents for the measurement of CRP were supplied by P.Z. Cormay, ul. Rapackiego 19, 20-150 Lublin, Poland, P.O. Box 122 20-954 Lublin 2.

Specimen Collection and Sample Storage

Serum may be used for measurement of CRP. Serum was collected from 5ml seroseparator vacutainers. Specimens were centrifuged on the day of venepuncture and divided into 1ml aliquots. Serum was either analysed immediately or stored at -40 to -80°C until there were sufficient samples to run a batch. The ADVIA 2400 uses 2 µl of sample in the test, although the minimum volume required in the specimen container was 200µl for the seroseparator vacutainers.

Method

The Cormay hs-CRP assay uses an anti-CRP antibody that has been sensitized to latex particles. The antibody coats the surface of latex particles forming a milky appearing sensitised latex. This antibody then reacts with CRP within the sample and results in visible agglutination. Latex particles are used to magnify the antigen-antibody complex. The degree of agglutination is detected as a decrease in the intensity of transmitted light at a wavelength of 572nm (turbidimetry), which is proportional to the amount of CRP within the sample. The concentration of CRP is determined by interpolation from a calibration curve prepared from calibrators of known concentration. The assay is analysed on the Siemens Advia 2400 (Cormay).

Sensitivity

The minimum detectable concentration of CRP is 0.01 mg/dL.

Quality Controls

The inter-assay CV was less than 10% and intra-assay CV was less than < 5%.

Full Methods for the measurement of inflammatory markers using biochip

Equipment

Inflammatory markers were measured using the Randox Evidence Investigator, manufactured by Randox Laboratories Ltd., Belfast, UK. Reagents for the measurement of IL-4, IL-6, IL-10, TNF-α, VEGF, IL-1β, IL-1RA, MCP-1 were supplied by Randox.

Specimen Collection and Sample Storage

Serum may be used for measurement of these inflammatory markers. Serum was collected from 5ml seroseparator vacutainers. Specimens were centrifuged on the day of venepuncture and divided into 1ml aliqots and stored at -40 to -80°C until there were sufficient samples to run a batch. The ADVIA 2400 uses 2 µl of sample in the test, although the minimum volume required in the specimen container was 200µl for the seroseparator vacutainers.

Method

The Randox Evidence Investigator is a biochip analyser which allows simultaneous detection of multiple analytes from a single sample. It uses the principle of enzyme-linked immunoabsorbent assay (ELISA). The samples are loaded onto the biochips and, after washing, a single set of reagent is added to the biochip before imaging. There are separate test regions for the respective markers on each biochip, chemiluminescence creates light at each of the test regions which is simultaneously detected by the Evidence Investigator.

Quality Controls

The inter-assay CV was less than 15% and intra-assay CV was less than < 10%.

Full Methods for the measurement of full blood cell count

Equipment

Reagents supplied by Siemens Medical Solutions Diagnostics Limited, Bayer House, Strawberry Hill, Newbery, Berks. RG14 1JA

Specimen Collection and Sample Storage

Whole blood specimens are required for measurement of FBC. EDTA is a suitable anticoagulants and were used for measurement of FBC for this study. Samples were collected in 3ml EDTA vacutainers. Samples were analysed on the day of venepuncture or stored at 4°C overnight before analysis. This is within the requirements for clinical measurement of FBC (storage at 4°C for up to one week at laboratory).

Method

The Siemens Advia 2120 is used to analyse the full blood count (FBC), Nucleated Red Blood Cell Count (NRBC) and Reticulocyte count. These are important tests requested by the clinician to evaluate the health of a patient. The results are used in conjunction with other diagnostic tests to diagnose, treat and monitor the progress of a patient. The Advia 2120 is a haematology analyser, which has the capacity to analyse the following test profiles: CBC/DIFF, CBC/DIFF/RETIC, CBC/RETIC.

The Siemens Advia 2120 uses; hydrodynamic focusing, to produce a single stream of cells; the peroxidase method, use of the myeloperoxidase enzyme to distinguish between the different white cell types (neutrophils, monocytes and eosinophils are peroxidase positive whereas lymphocytes and basophils are peroxidase negative); the basophil method, addition of phyhalic acid to lyse all cells and platelets except basophils; cyanide free haemoglobin detection, to measure haemoglobin.

Full Methods for the measurement of adiponectin

Equipment

Quantikine Adiponectin ELISA kit distributed by R & D Systems Europe, 19 Barton Lane, Abingdon Science Park, Abingdon, Oxon, OX14 3NB.

Specimen Collection and Sample Storage

Serum may be used for measurement of adiponectin. Serum was collected from 5ml seroseparator vacutainers. Specimens were centrifuged on the day of venepuncture and divided into 1ml aliqots and stored at -40 to -80°C until there were sufficient samples to run a batch. 50µl of sample in the test, although the minimum volume required in the specimen container was 200µl for the seroseparator vacutainers.

Method

The assay contains NSO-expressed recombinant human Adiponectin and has been shown to accurately quantitate the recombinant factor and measures total (low, middle, and high molecular weight) adiponectin.

The assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Adiponectin is pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Adiponectin present is bound by the immobilised antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for Adiponectin is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and colour develops in proportion to the amount of Adiponectin bound in the initial step. The colour development is stopped and the intensity of the colour is measured.

Data Collection Clinical Record Forms (CRFs) and Questionnaires

Baseline CRF

SOUL-D

Data collection schedule

SOUL-D ID No.: ☐☐☐☐

Researcher ID: ☐☐

Date of entry into study: __ __/ __ __/ __ __

0.1 Participant NHS number: ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

0.2 GP national code number: ☐ ☐ ☐ ☐ ☐

0.3 GP surgery: ……

0.4 GP address:……..

0.5 Laboratory site (bloods etc), please tick:
☐[1] KCH ☐[2] GSTT ☐[3] PRU ☐[4] UHL ☐[5] QMS ☐[6] Mayday
☐[7] Other……..

0.6 GP borough
☐[1] Lambeth ☐[2] Southwark ☐[3] Lewisham ☐[4] Bromley
☐[5] Other……

Entry Visit Checklist

PIS given to participant	☐ Yes	☐ No
Original consent to study files	☐ Yes	☐ No
Copy of consent to participant	☐ Yes	☐ No
Copy of consent to GP	☐ Yes	☐ No
0.7 Baseline questionnaire completed	☐[1] Yes	☐[2] No
0.8 Baseline bloods completed	☐[1] Yes	☐[2] No
0.9 Baseline CRF completed	☐[1] Yes	☐[2] No

0.10 Researcher Notes

a. Questionnaire booklet read to subject?	☐[1] Yes	☐[2] No

b. If yes, because of: ☐[1] Vision problem ☐[2] Literacy ☐[3] Other ……

0.11 Year 1 Visit Checklist

a. Consent reviewed with participant:
☐[1]Yes ☐[2]Refused ☐[3]Non-contactable

b. Questionnaire completed	☐[1] Yes	☐[2] No
c. Year 1 bloods completed	☐[1] Yes	☐[2] No
d. Year 1 CRF completed	☐[1] Yes	☐[2] No

0.12 Year 2 Visit Checklist

a. Consent reviewed with participant:
☐[1]Yes ☐[2]Refused ☐[3]Non-contactable

b. Questionnaire completed	☐[1] Yes	☐[2] No
c. Year 2 bloods completed	☐[1] Yes	☐[2] No
d. Year 2 CRF completed	☐[1] Yes	☐[2] No

1.0 Date of data collection
___ ___ / ___ ___ / ___ ___ (dd/mm/yy)

1. Socio-demographics data

1.1 Date of birth
___ ___ / ___ ___ / ___ ___ (dd/mm/yy)

1.2 Gender

☐[1] Male
☐[2] Female

1.3 What is your legal partnership status?
Please tick the box that indicates your legal partnership status.

☐[1] Married
☐[2] Cohabitating
☐[3] Separated
☐[4] Divorced
☐[5] Widowed
☐[6] Single

1.4 Have you had children?
☐[1] Yes *If yes, please list how many_____*
☐[2] No

1.5 What is your ethnic group?
Choose ONE section from a. to e., then tick the appropriate box to indicate your ethnic group.

a. White
☐[1] British
☐[2] Irish
☐[3] Any Other White background, *please write in*
……

b. Mixed
☐[4] White and Black Caribbean
☐[5] White and Black African
☐[6] White and Asian
☐[7] Any Other Mixed background, *please write in*
……..

c. Asian or Asian British
☐[8] Indian
☐[9] Pakistani
☐[10] Bangladeshi
☐[11] Any Other Asian background, *please write in*
……..

d. Black or Black British
☐[12] Caribbean

☐[13] African
☐[14] Any Other Black background, *please write in*
……...

e. Chinese or other ethnic group
☐[15] Chinese
☐[16] Any Other, *please write in*
……

1.6 What is your country of birth?

☐[1] England
☐[2] Elsewhere, *please write in the present name of the country*
……

1.7 Do you consider yourself to have a faith or religious identity?
☐[1] Yes
☐[2] No

1.8 Do you attend a formal congregation or religious group?
☐[1] Yes
If yes, list group _____
☐[2] No

1.9 Employment status

Are you currently….

☐[1] In full-time employment
☐[2] In part-time employment
☐[3] On sick leave
☐[4] Unemployed
☐[5] Medically retired
☐[6] A housewife/husband
☐[7] Retired

The following questions refer to your current main job, or (if you are not working now) to your last main job. Please tick one box only per question.

1.10 Employee or self-employed
Do (did) you work as an employee or are (were) you self-employed?
☐[1] Employee
☐[2] Self-employed with employees
☐[3] Self-employed / freelance without employees (go to question 1.13)

1.11 Number of employees
For employees: Indicate below how many people work (worked) for your employer at the place where you work (worked).

For self-employed: Indicate below how many people you employ (employed). Go to question 1.13 when you have completed this question.

Please tick box.

☐[1] 1 to 24
☐[2] 25 or more

1.12 Supervisory status
Do (did) you supervise any other employees?
A supervisor or foreman is responsible for overseeing the work of other employees on a day-to-day basis.

☐[1] Yes
☐[2] No

1.13 Occupation
Please tick one box to show which **best** describes the sort of work you do. (If you are not working now, please tick a box to show what you did in your last job).

PLEASE TICK ONE BOX ONLY.

☐[1] **Modern professional occupations**
Such as: teacher, nurse, physiotherapist, social worker, welfare officer, artist, musician, police officer (sergeant or above), software designer

☐[2] **Clerical and intermediate occupations**
Such as: secretary, personal assistant, clerical worker, office clerk, call centre agent, nursing auxiliary, nursery nurse

☐[3] **Senior mangers or administrators**
(usually responsible for planning, organising, and co-ordinating work and for finance)
Such as: finance manager, chief executive

☐[4] **Technical and craft occupations**
Such as: motor mechanic, fitter, inspector, plumber, printer, tool maker, electrician, gardener, train driver

☐[5] **Semi-routine manual and service occupations**
Such as: postal worker, machine operative, security guard, caretaker, farm worker, catering assistant, receptionist, sales assistant

☐[6] **Routine manual and service occupations**
Such as: HGV driver, van driver, cleaner, porter, packer, sewing machinist, messenger, labourer, waiter / waitress, bar staff

☐[7] **Middle or junior managers**
Such as: office manager, retail manager, bank manager, restaurant manager, warehouse manager, publican

☐[8] **Traditional professional occupations**
Such as: accountant, solicitor, medical practitioner, scientist, civil / mechanical engineer

1.14 What is your current (or last) job title?
Please write in: …….

1.15 Do you drive/hold current driving licence?
☐[1] Yes
☐[2] No

2. Diabetes history

2.1 Date of T2DM diagnosis
___ ___ / ___ ___ (mm/yy)

Diabetes Presentation:

2.2 Was participant admitted to hospital when they were diagnosed with T2DM?
☐[1] Yes
☐[2] No

2.3 Mode of Onset:

☐[1] Diabetic Symptoms
☐[2] Routine/screening blood or urine test and symptoms
☐[3] Routine/screening blood or urine test, no symptoms
☐[4] Ketoacidosis (proven) i.e. African patients
☐[5] Non-ketotic hyperosmolar (proven)
☐[6] Ketones present (not DKA)
☐[7] Diagnosed during pregnancy
☐[8] Not known

2.3 Have you attended structured diabetes education? (e.g. DESMOND)

☐[1] Yes
if yes, date (mm/yy)____/____
☐[2] No
☐[3] Waiting list

2.4 Physical examination **at diagnosis**:

Measurement	Units	Value	Month/year (mm/yy)
a. Height	cm		
b. Weight	kg		
c. BMI	wt/ht^2		
d. Blood pressure systolic	mmHg		
e. Blood pressure diastolic	mmHg		

2.5 Lab tests **at diagnosis**:

Test	Units	Value	Month/year (mm/yy)
a. Triglycerides	mmol/L		
b. LDL	mmol/L		
c. HDL	mmol/L		
d. Total cholesterol	mmol/L		
e. HbA1c	%		

2.6 Complications screening **at diagnosis**

Kidney:

2.6.1 Microalbuminuria (ACR):

a. Sample collection:
☐1 Data not available (continue to 2.6.2)
☐2 Early morning urine
☐3 Random sample
☐4 Not indicated

b. Results:
☐1 Negative (ACR < 3)
☐2 Positive (ACR ≥ 3)

2.6.2 Proteinuria (urine dipstick)

a. Sample Collection:
☐1 Data not available (continue to 2.6.3)
☐2 Early morning urine
☐3 Random sample
☐4 Not indicated

b. Results:
☐1 Negative (0 – trace on urine dipstick)
☐2 Positive (1+ - 3+ on urine dipstick)

Eyes:

Retinopathy
2.6.3 Attended DECS
☐[1] Yes
☐[2] No
☐[3] Appointment booked *(date:* ___ ___ /___ ___ / ___ ___ (dd/mm/yy))
☐[4] No appointment booked

2.6.4 DECS coding
☐[1] No retinopathy
☐[2] Treated retinopathy
(laser, photocoagulation, vitrectomy, quiescent retinopathy)
☐[3] Non-sight threatening retinopathy
(background, mild/minimal pre-proliferative, mild/moderate non-proliferative)
☐[4] Sight-threatening retinopathy
(maculopathy, moderate/severe pre-proliferative, pre-proliferative and maculopathy, non-proliferative maculopathy, at risk of and with clinically significant macula oedema)

2.6.4 Date of DECS assessment: ___ ___ / ___ ___ (mm/yy)

2.6.5 Laser treatment
☐[1] Yes
If yes, date: ___ ___ / ___ ___ (mm/yy)
☐[2] No

2.6.6 Cataracts
☐[1] Yes
☐[2] No

2.6.6 Registered partially sighted, secondary to diabetes
☐[1] Yes
If yes, date: ___ ___ / ___ ___ (mm/yy)
☐[2] No

Feet:

Foot ulcers/history of ulcer
2.6.7 R foot:
☐[1] Yes
☐[2] No
☐[3] Healed

2.6.8 L foot:
☐[1] Yes
☐[2] No
☐[3] Healed

Macrovascular disease

2.6.9 Myocardial infarction (MI) / Heart attack
☐[1] Yes
☐[2] No

2.6.10 Coronary angioplasty/CABG
☐[1] Yes
☐[2] No

2.6.11 Cerebral vascular accident (CVA) / Stroke
☐[1] Yes
☐[2] No

2.6.12 Carotid revascularisation
☐[1] Yes
If yes, date: ___ ___ / ___ ___ (mm/yy)
☐[2] No

2.6.13 Limb revascularisation
☐[1] Yes
If yes, date: ___ ___ / ___ ___ (mm/yy)
☐[2] No

2.6.14 Amputation
☐[1] Yes
If yes, date: ___ ___ / ___ ___ (mm/yy)
If yes: ☐[2] Major
☐[1] Minor
☐[2] No

2.6.15 Erectile dysfunction (see recruiter booklet for explanation)
☐[1] Yes
☐[2] No

Hypoglycaemia:

2.6.16 Severe hypoglycaemia (needing 3rd party assistance, see recruiter booklet for explanation)

☐[1] Yes
☐[2] No

2.6.17 If yes how many episodes in last 12 months? ☐☐☐

2.7 QOF psychological assessment **at diagnosis**:

2.7.1 Completion of QOF 2-item depression screening
☐[1] Yes
If yes, date: ___ ___ / ___ ___ (mm/yy)
☐[2] No

2.7.2 Results: Positive depression screen
☐[1] Yes
☐[2] No *(if no, go to section 3)*

2.7.3 If positive screen, full depression screen completed? (e.g. PHQ-9, HADS)
☐[1] Yes
If yes, date: ___ ___ / ___ ___ (mm/yy)
☐[2] No

2.7.4 If positive screen, any management for depression?
☐[1] Self-help (e.g. book/leaflet)
☐[2] Anti-depressant
☐[3] Counselling
☐[4] CBT
☐[5] Diabetes specific psychological treatment (e.g. MET/MI for diabetes)
☐[6] No treatment

3. Current depression

Depression (CIS-R)
Interviewer: please tick box
3.1 Have you had a spell of feeling sad, miserable or depressed in the past month?
☐[1] Yes
☐[2] No

3.2 During the past month, have you been able to enjoy or take an interest in things as much as you usually do?
☐[1] Yes
☐[2] No

3.3 History of depressive illness?
☐[1] Yes
☐[2] No

4. Current physical/psychological status (QOF screen=grey boxes)
Date of assessment _ _/_ _/_ _

Physical examination:

	Units	**Value**
a. Height	cm	
b. Seated height	cm	
c. Leg length	difference a-b	
d. Weight	kg	
e. BMI	wt/ht^2	
f. Waist circumference	cm	
g. Blood pressure systolic	mmHg	
h. Blood pressure diastolic	mmHg	

Neuropathy assessment:

	Units	**Value**
Vibration Perception Threshold		
4.1 **R 1st toe**	volts	
4.2 **L 1st toe**	volts	
10g monofilament sensation		
Test 5 sites: 1st, 2nd, 3rd, & 5th plantar metatarsal heads and plantar aspect of great toe. If feels < 3/5, abnormal result.		
4.1 **R foot**	no. of sites	/5
4.2 **L foot**	no. of sites	/5

Foot pulses:

4.3 **R foot** dorsalis pedis	☐1 Present ☐2 Absent
4.4 **R foot** posterior tibial	☐1 Present ☐2 Absent
4.5 **L foot** dorsalis pedis	☐1 Present ☐2 Absent
4.6 **L foot** posterior tibial	☐1 Present ☐2 Absent

Depression screening:

Low mood – use answer from 3.1	☐1 Yes ☐2 No
Loss of interest in activities – use answer from 3.2	☐1 Yes ☐2 No
4.9 Positive screen (if yes to either of the above)	☐1 Yes ☐2 No
4.10 PHQ-9 score from participant questionnaire	

5. Current cognitive status

Telephone Interview for Cognitive Status (TICS-M)
Orientation
5.1 What day of the week is it?
Day
☐[1] Correct
☐[2] Incorrect

5.2 What is today's date? (day/month/year)
Day
☐[1] Correct
☐[2] Incorrect

Month
☐[1] Correct
☐[2] Incorrect

Year
☐[1] Correct
☐[2] Incorrect

5.3 What season are we in?
Season
☐[1] Correct
☐[2] Incorrect

5.4 What is your age?
☐[1] Correct
☐[2] Incorrect

5.5 What is your telephone number (including code)?
Code + number
☐[1] Correct
☐[2] Incorrect

Registration/Free recall
5.6 I'm going to read you a list of 10 words. Please listen carefully and try to remember them. When I am done, tell me as many as you can in any order. Ready?

(Read words from list below).

Now, tell me all the words you can remember.
Yes No
☐[1] ☐[2] Cabin
☐[1] ☐[2] Pipe
☐[1] ☐[2] Elephant
☐[1] ☐[2] Chest
☐[1] ☐[2] Silk
☐[1] ☐[2] Theatre
☐[1] ☐[2] Watch
☐[1] ☐[2] Whip
☐[1] ☐[2] Pillow
☐[1] ☐[2] Giant

Attention/Calculation
5.7 Please take 7 away from 100
Answer: 93
☐[1] Correct
☐[2] Incorrect

Now continue to take 7 away from what you have left over until I ask you to stop.
Answer: 86
☐[1] Correct
☐[2] Incorrect

Answer: 79
☐[1] Correct
☐[2] Incorrect

Answer: 72
☐[1] Correct
☐[2] Incorrect

Answer: 65
☐[1] Correct
☐[2] Incorrect

5.8 Please count backwards from 20 to 1.
No mistakes?
☐[1] Yes
☐[2] No

Comprehension, semantic & recent memory
5.9 What do people usually use to cut paper?
Answer: Scissors
☐[1] Correct
☐[2] Incorrect

5.10 What is the prickly green plant found in the desert?
Answer: Cactus
☐[1] Correct
☐[2] Incorrect

5.11 Who is the reigning monarch?
Answer: Elizabeth, Queen Elizabeth, Queen Elizabeth the 2nd
☐[1] Correct
☐[2] Incorrect

5.12 Who is the Prime Minister now?
Answer: Gordon Brown (if changed write in here_____)
☐[1] Correct
☐[2] Incorrect

5.13 What is the opposite of East?
Answer: West
☐[1] Correct
☐[2] Incorrect

Language/repetition
5.14 Please say this, 'Methodist Episcopal'.
Was this pronounced exactly right?
☐[1] Yes
☐[2] No

5.15 Please repeat the list of 10 words I read earlier.
Yes No
☐[1] ☐[2] Cabin
☐[1] ☐[2] Pipe
☐[1] ☐[2] Elephant
☐[1] ☐[2] Chest
☐[1] ☐[2] Silk
☐[1] ☐[2] Theatre
☐[1] ☐[2] Watch
☐[1] ☐[2] Whip
☐[1] ☐[2] Pillow
☐[1] ☐[2] Giant

NART

Interviewer:

I want you to read slowly down this list of words starting here. *(Hand patient NART word list and indicate CHORD).* After each word please wait until I say 'next' before reading the next word. I must warn you that there are many words that you probably won't recognise; in fact most people don't know them, so just have a guess at these, O.K.? Go ahead:

Column 1

5.17 CHORD (körd)
☐[1] Correct
☐[2] Incorrect

5.18 ACHE (āk)
☐[1] Correct
☐[2] Incorrect

5.19 DEPOT (dep'ō)
☐[1] Correct
☐[2] Incorrect

5.20 AISLE (īl)
☐[1] Correct
☐[2] Incorrect

5.21 BOUQUET (bōōk'ā, bōōkā', bōkā')
☐[1] Correct
☐[2] Incorrect

5.22 PSALM (säm)
☐[1] Correct
☐[2] Incorrect

5.23 CAPON (kā'pn)
☐[1] Correct
☐[2] Incorrect

5.24 DENY (di-nī)
☐[1] Correct
☐[2] Incorrect

5.25 NAUSEA (nö'si-ə, nö'zhə)
☐[1] Correct
☐[2] Incorrect

5.26 DEBT (det)
☐[1] Correct
☐[2] Incorrect

5.27 COURTEOUS (kûrt'yəs)
☐[1] Correct
☐[2] Incorrect

5.28 RAREFY (rār'i-fī)
☐[1] Correct
☐[2] Incorrect

5.29 EQUIVOCAL (i-kwiv'ə-kl)
☐[1] Correct
☐[2] Incorrect

5.30 NAÏVE (nä-ēv)
☐[1] Correct
☐[2] Incorrect

5.31 CATACOMB (kat'ə'kōōm)
☐[1] Correct
☐[2] Incorrect

5.32 GAOLED (jāld)
☐[1] Correct
☐[2] Incorrect

5.33 THYME (tīm)
☐[1] Correct
☐[2] Incorrect

5.34 HEIR (àr)
☐[1] Correct
☐[2] Incorrect

5.35 RADIX (rā'diks)
☐[1] Correct
☐[2] Incorrect

5.36 ASSIGNATE (as'-ig-nāt)
☐[1] Correct
☐[2] Incorrect

5.37 HIATUS (hī-ā'təs)
☐[1] Correct
☐[2] Incorrect

5.38 SUBTLE (sut'l)
☐[1] Correct
☐[2] Incorrect

5.39 PROCREATE (prō'kri-àt)
☐[1] Correct
☐[2] Incorrect

5.40 GIST (jist)
☐[1] Correct
☐[2] Incorrect

5.41 GOUGE (gowj)
☐[1] Correct
☐[2] Incorrect

Column 2
5.42 SUPERFLUOUS (sōō-pûr'flŏŏ-əs, sū-pûr'flŏŏ-əs)
☐[1] Correct
☐[2] Incorrect

5.43 SIMILE (sim'i-li)
☐[1] Correct
☐[2] Incorrect

5.44 BANAL (bən-al')
☐[1] Correct
☐[2] Incorrect

5.45 QUADRUPED (kwod'rŏŏ-ped)
☐[1] Correct
☐[2] Incorrect

5.46 CELLIST (chel'ist)
☐[1] Correct
☐[2] Incorrect

5.47 FAÇADE (fa-säd')
☐[1] Correct
☐[2] Incorrect

5.48 ZEALOT (zel'ət)
☐[1] Correct
☐[2] Incorrect

5.49 DRACHM (dram)
☐[1] Correct
☐[2] Incorrect

5.50 AEON (ē'on)
☐[1] Correct
☐[2] Incorrect

5.51 PLACEBO (plə-sē'bō)
☐1 Correct
☐2 Incorrect

5.52 ABSTEMIOUS (ab-stē'mi-əs)
☐1 Correct
☐2 Incorrect

5.53 DÉTENTE (dā-tát)
☐1 Correct
☐2 Incorrect

5.54 IDYLL (id'il, id'əl)
☐1 Correct
☐2 Incorrect

5.55 PUERPERAL (pū-ûr'pər-əl)
☐1 Correct
☐2 Incorrect

5.56 AVER (ə-vûr')
☐1 Correct
☐2 Incorrect

5.57 GAUCHE (gō sh)
☐1 Correct
☐2 Incorrect

5.58 TOPIARY (tō'pi-ə-ri)
☐1 Correct
☐2 Incorrect

5.59 LEVIATHIAN (le-vi'ə-thən)
☐1 Correct
☐2 Incorrect

5.60 BEATIFY (bi-at'i-fi)
☐1 Correct
☐2 Incorrect

5.61 PRELATE (prel'it)
☐1 Correct
☐2 Incorrect

5.62 SIDEREAL (sī-dē'ri-əl)
☐1 Correct
☐2 Incorrect

5.63 DEMESNE (di-mān, di-mēn)
☐[1] Correct
☐[2] Incorrect

5.64 SYNCOPE (sing'kə-pē)
☐[1] Correct
☐[2] Incorrect

5.65 LABILE (lā'bil)
☐[1] Correct
☐[2] Incorrect

5.66 CAMPANILE (kam-pan-ē'lā, kam-pan-ē'lē)
☐[1] Correct
☐[2] Incorrect

6. Medication Review / Current Treatment

6.1 Herbal Remedies
☐[1] Yes
☐[2] No

Name	Total daily dose	Units
a.		
b.		
c.		
d.		
e.		
f.		

6.2 Diabetes tablets
☐[1] Yes
☐[2] No

Name	Total daily dose	Units
a. Metformin (Glucophage)		
b. Acarbose		
c. Repaglinide (Prandin)		
d. Nateglinide (Starlix)		
e. Glibenclamide (Daomil, Euglucon)		
f. Gliclazide (Diamicron)		
g. Glimepiride (Amaryl)		
h. Glipizide (Glibenese, Minodiab)		
i. Other *Name:*		

6.3 Insulin
☐[1] Yes
☐[2] No

Name	Dose (Units)	Frequency
a. Insulin Lispro (Humalog)		☐ Once daily ☐ Twice daily ☐ Three times daily ☐ Other:
b. Insulin Aspart (NovoRapid)		☐ Once daily ☐ Twice daily ☐ Three times daily ☐ Other:
c. Insulin Glulisine (Apidra)		☐ Once daily ☐ Twice daily ☐ Three times daily ☐ Other:
d. Insulin Glargine (Lantus)		☐ Once daily ☐ Twice daily ☐ Three times daily ☐ Other:
e. Isophane Insulin (NPH) (eg. Insulatard, Humulin I)		☐ Once daily ☐ Twice daily ☐ Three times daily ☐ Other:
f. Soluble Insulin (eg. Humulin S, Actrapid)		☐ Once daily ☐ Twice daily ☐ Three times daily ☐ Other:
g. Insulin Detemir (Levemir)		☐ Once daily ☐ Twice daily ☐ Three times daily ☐ Other:
h. Humalog Mix 25		☐ Once daily ☐ Twice daily ☐ Three times daily ☐ Other:
i. Humalog Mix 50		☐ Once daily ☐ Twice daily ☐ Three times daily ☐ Other:
j. NovoMix 30 (eg. Mixtard 30)		☐ Once daily ☐ Twice daily ☐ Three times daily ☐ Other:
k. Other *Name:*		☐ Once daily ☐ Twice daily ☐ Three times daily ☐ Other:

6.4 Cholesterol – lowering medications
☐[1] Yes
☐[2] No

Name	Total daily dose	Units
a. Atvorvastatin (Lipitor)		
b. Simvastatin (Zocor)		
c. Bezafibrate (Bezalip)		
d. Fenofibrate (Lipantil)		
e. Colestyramine (Questran)		
f. Ezetumibe		
g. Other *Name:*		

6.5 Anti-hypertensives
☐[1] Yes
☐[2] No

Name	Total daily dose	Units
a. Amlodopine		
b. Ramipril (Lopace)		
c. Doxazosin		
d. Felodipine		
e. Labetalol		
f. Atenolol		
g. Other *Name:*		

6.6 Diuretics
☐[1] Yes
☐[2] No

Name	Total daily dose	Units
a. Bendroflumethiazide		
b. Furosemide		
c. Spirinolactone		
d. Hydrochlorathiazide		
e. Other *Name:*		

6.7 NSAIDS and Opiods
☐[1] Yes
☐[2] No

Name	Total daily dose	Units
a. Aspirin		
b. Ibruprofen		
c. Codeine		
d. Hydromorphone (Dilaudid)		
e. Meperidine		
f. Oxycodone		
g. Other *Name:*		

6.8 Other medications

☐[1] Yes

☐[2] No

Name	Total daily dose	Units
a.		
b.		
c.		
d.		
e.		

7. SOUL-D Lab Tests

Date obtained: ___ ___/ ___ ___ (mm/yy)

Lab: ☐[1] KCH ☐[2] GSTT ☐[3] PRU ☐[4] UHL ☐[5] QMS ☐[6]Mayday ☐[7] Other……..

Test	Units	Value	Ref. range
Lipids			
a. Triglycerides	mmol/L		
b. LDL	mmol/L		
c. HDL	mmol/L		
d. Total cholesterol	mmol/L		
LFTs			
e. ALT (alanine aminotransferase)	IU/L		
f. AST (aspartate aminotransferase)	IU/L		
g. ALP (alkaline phosphatase)	IU/L		30-130
h. GGT (gamma-glutamyl transferase)	IU/L		1-55
Renal			
i. Creatinine	umol/L		45-120
j. eGFR	ml/min		
FBC			
k. Total white count (WBC)	10\S\9/l		4.00-11.00
l. Haemoglobin (Hb)	g/dl		11.5-15.5
m. Platelet count (PLT)	10\S\9/l		150-450
n. Neutrophils	10\S\9/l		0.20-6.30
o. Lymphocytes	10\S\9/l		1.30-4.00
TFTs			
p. Thyroid stimulating hormone (TSH)	mU/L		0.30-5.50)
q. Free thyroxine	pmol/L		9.0-25.0
r. HbA1c	%		
s. Prolactin	mU/L		< 510mU
t. C-reactive Protein	mg/l		< 5.0
u. Cortisol	nmol/L		130-580
v. Insulin levels	mU/L		4.4-26.0
u. Plasma glucose (fasting)	mmol/L		3.0-6.0
w. HOMA-IR (v x u)/22.5			
x. ACR	µg/mg		

12-Month CRF

12 months

SOUL-D ID No.: □□□□

Researcher ID: □□

Date of 12-month follow-up: __ __/ __ __/ __ __

0.1 Year 1 Visit Checklist

a. Consent reviewed with participant:

☐[1]Yes ☐[2]Withdrawn ☐[3]Non-contactable ☐[4] Dead

b. Questionnaire completed ☐[1] Yes ☐[2] No

c. Year 1 bloods completed ☐[1] Yes ☐[2] No

d. Year 1 CRF completed ☐[1] Yes ☐[2] No

0.2 Withdrawals, non-contactables, deaths:

a. Withdrawn Date __ __/__ __/__ __

NB: please complete sections of the CRF using GP records and ensure data manager aware so that no further study letters sent out

b. Non-contactable

Date last seen at GP practice __ __/__ __/__ __

NB: please check dates of last prescription uptake as well as general records and complete sections of the CRF using GP records

c. If dead

Date __ __/__ __/__ __ Cause ……..

d. Main cause of death:

Non-specific CVD ☐[1]

MI ☐[2]

CVA ☐[3]

Infection ☐[4]

Cancer ☐[5]

Renal failure ☐[6]

Complications from liver disease ☐[7]

NB: please complete sections of the CRF using GP records

1. Socio-demographics data

1.1 What is your legal partnership status?
Please tick the box that indicates your legal partnership status.

☐[1] Married
☐[2] Cohabitating
☐[3] Separated
☐[4] Divorced
☐[5] Widowed
☐[6] Single

1.2 Employment status

Are you currently….

☐[1] In full-time employment
☐[2] In part-time employment
☐[3] On sick leave
☐[4] Unemployed
☐[5] Medically retired
☐[6] A housewife/husband
☐[7] Retired

The following questions refer to your current main job, or (if you are not working now) to your last main job. Please tick one box only per question.

1.3 Employee or self-employed
Do (did) you work as an employee or are (were) you self-employed?
☐[1] Employee
☐[2] Self-employed with employees
☐[3] Self-employed / freelance without employees (go to question 1.6)

1.4 Number of employees
For employees: Indicate below how many people work (worked) for your employer at the place where you work (worked).

For self-employed: Indicate below how many people you employ (employed). Go to question 1.13 when you have completed this question.

Please tick box.

☐[1] 1 to 24
☐[2] 25 or more

1.5 Supervisory status
Do (did) you supervise any other employees?
A supervisor or foreman is responsible for overseeing the work of other employees on a day-to-day basis.

☐[1] Yes
☐[2] No

1.6 Occupation
Please tick one box to show which **best** describes the sort of work you do. (If you are not working now, please tick a box to show what you did in your last job).

PLEASE TICK ONE BOX ONLY.

☐[1] **Modern professional occupations**
Such as: teacher, nurse, physiotherapist, social worker, welfare officer, artist, musician, police officer (sergeant or above), software designer

☐[2] **Clerical and intermediate occupations**
Such as: secretary, personal assistant, clerical worker, office clerk, call centre agent, nursing auxiliary, nursery nurse

☐[3] **Senior mangers or administrators**
(usually responsible for planning, organising, and co-ordinating work and for finance)
Such as: finance manager, chief executive

☐[4] **Technical and craft occupations**
Such as: motor mechanic, fitter, inspector, plumber, printer, tool maker, electrician, gardener, train driver

☐[5] **Semi-routine manual and service occupations**
Such as: postal worker, machine operative, security guard, caretaker, farm worker, catering assistant, receptionist, sales assistant

☐[6] **Routine manual and service occupations**
Such as: HGV driver, van driver, cleaner, porter, packer, sewing machinist, messenger, labourer, waiter / waitress, bar staff

☐[7] **Middle or junior managers**
Such as: office manager, retail manager, bank manager, restaurant manager, warehouse manager, publican

☐[8] **Traditional professional occupations**
Such as: accountant, solicitor, medical practitioner, scientist, civil / mechanical engineer

1.7 What is your current (or last) job title?
Please write in: …….

1.8 If not working are you in full-time education?
☐[1] Yes
☐[2] No

1.9 Do you drive/hold current driving licence?
☐[1] Yes
☐[2] No

2.0 Diabetes complications screening at 12 months

Kidney:

2.1 Microalbuminuria (ACR):

a. Sample collection:
☐[1] Data not available (continue to 2.6.2)
☐[2] Early morning urine
☐[3] Random sample
☐[4] Not indicated

b. Results:
☐[1] Negative (ACR < 3)
☐[2] Positive (ACR ≥ 3)

2.2 Proteinuria (urine dipstick)

a. Sample Collection:
☐[1] Data not available (continue to 2.6.3)
☐[2] Early morning urine
☐[3] Random sample
☐[4] Not indicated

b. Results:
☐[1] Negative (0 – trace on urine dipstick)
☐[2] Positive (1+ - 3+ on urine dipstick)

Eyes:

Retinopathy
2.3 Attended DECS
☐[1] Yes
☐[2] No
☐[3] Appointment booked *(date:* ___ ___ /___ ___ / ___ ___ (dd/mm/yy))
☐[4] No appointment booked

2.4 DECS coding
☐[1] No retinopathy
☐[2] Treated retinopathy
(laser, photocoagulation, vitrectomy, quiescent retinopathy)
☐[3] Non-sight threatening retinopathy
(background, mild/minimal pre-proliferative, mild/moderate non-proliferative)
☐[4] Sight-threatening retinopathy
(maculopathy, moderate/severe pre-proliferative, pre-proliferative and maculopathy, non-proliferative maculopathy, at risk of and with clinically significant macula oedema)

2.5 Date of last DECS assessment: ___ ___ / ___ ___ (mm/yy)

2.6 Laser treatment
☐[1] Yes
If yes, date: ___ ___ / ___ ___ (mm/yy)
☐[2] No

2.7 Cataracts
☐[1] Yes
☐[2] No

2.8 Registered partially sighted, secondary to diabetes
☐[1] Yes
If yes, date: ___ ___ / ___ ___ (mm/yy)
☐[2] No

Feet:

Foot ulcer since baseline visit
2.9 R foot:
☐[1] Yes
☐[2] No
☐[3] Healed

2.10 L foot:
☐[1] Yes
☐[2] No
☐[3] Healed

Macrovascular disease since baseline visit
2.12 Myocardial infarction (MI) / Heart attack
☐[1] Yes
☐[2] No

2.13 Coronary angioplasty/CABG
☐[1] Yes
☐[2] No

2.14 Cerebral vascular accident (CVA) / Stroke
☐[1] Yes
☐[2] No

2.15 Carotid revascularisation
☐[1] Yes
If yes, date: ___ ___ / ___ ___ (mm/yy)
☐[2] No

2.16 Limb revascularisation
☐[1] Yes
If yes, date: ___ ___ / ___ ___ (mm/yy)
☐[2] No

2.17 Amputation
☐[1] Yes
If yes, date: ___ ___ / ___ ___ (mm/yy)
If yes: ☐[2] Major
☐[1] Minor
☐[2] No

2.18 Erectile dysfunction (see recruiter booklet for explanation)
☐[1] Yes
☐[2] No

Hypoglycaemia:
2.19 Severe hypoglycaemia (needing 3rd party assistance, see recruiter booklet for explanation)

☐[1] Yes
☐[2] No

2.20 If yes how many episodes in last 12 months? ☐☐☐

Family history of Type 2 or gestational diabetes:
2.21 1st degree relatives (do not record step family only blood relations)

	Yes[1]	No[2]	Not known[3]	Number (c, d, e only)
a. Mother				
b. Father				
c. Brother (n)				
d. Sister (n)				
e. Child (n)				

2.22 1st degree relatives with diabetes

	Diabetes?			If yes			
	Yes	No	Not known	Diagnosed <50years	50-70 years	71 years+	Not known
a. Mother							
b. Father							
c. Brother (n)							
d. Sister (n)							
e. Child (n)							

2.23 1st degree relatives who are obese

Obese? (BMI 30+)			
	Yes	No	Not known
a. Mother			
b. Father			
c. Brother (n)			
d. Sister (n)			
e. Child (n)			

3. Current depression

Depression (CIS-R)
Interviewer: please tick box
3.1 Have you had a spell of feeling sad, miserable or depressed in the past month?
☐[1] Yes
☐[2] No

3.2 During the past month, have you been able to enjoy or take an interest in things as much as you usually do?
☐[1] Yes
☐[2] No

3.3 Any management for depression since baseline?
☐[1] Self-help (e.g. book/leaflet)
☐[2] Anti-depressant
☐[3] Counselling
☐[4] CBT
☐[5] Diabetes specific psychological treatment (e.g. MET/MI for diabetes)
☐[6] No treatment
☐[7] Not applicable (not depressed)

3.4 Does anyone in your family suffer from depression?

☐[1] Mother
☐[2] Father
☐[3] Brother
☐[4] Sister
☐[5] Spouse/partner
☐[6] Children
☐[7] No family history
☐[8] Not known

4. Current physical/psychological status (QOF screen=grey boxes)
Date of assessment

Physical examination:

	Units	**Value**
a. Height	cm	
b. Seated height	cm	
c. Leg length	difference a-b	
d. Weight	kg	
e. BMI	wt/ht^2	
f. Waist circumference	cm	
g. Blood pressure systolic	mmHg	
h. Blood pressure diastolic	mmHg	

Neuropathy assessment:

	Units	**Value**
Vibration Perception Threshold		
4.1 **R 1st toe**	volts	
4.2 **L 1st toe**	volts	
10g monofilament sensation		
Test 5 sites: 1st, 2nd, 3rd, & 5th plantar metatarsal heads and plantar aspect of great toe. If feels < 3/5, abnormal result.		
4.1 **R foot**	no. of sites	**/5**
4.2 **L foot**	no. of sites	**/5**

Foot pulses:

4.3 **R foot** dorsalis pedis	☐1 Present ☐2 Absent
4.4 **R foot** posterior tibial	☐1 Present ☐2 Absent
4.5 **L foot** dorsalis pedis	☐1 Present ☐2 Absent
4.6 **L foot** posterior tibial	☐1 Present ☐2 Absent

Depression screening:

Low mood – use answer from 3.1	☐1 Yes ☐2 No
Loss of interest in activities – use answer from 3.2	☐1 Yes ☐2 No
4.9 Positive screen (if yes to either of the above)	☐1 Yes ☐2 No
4.10 PHQ-9 score from participant questionnaire	

4.11 Blood glucose level		mmols/L

5. Current cognitive status

Telephone Interview for Cognitive Status (TICS-M)
Orientation
5.1 What day of the week is it?
Day
☐[1] Correct
☐[2] Incorrect

5.2 What is today's date? (day/month/year)
Day
☐[1] Correct
☐[2] Incorrect

Month
☐[1] Correct
☐[2] Incorrect

Year
☐[1] Correct
☐[2] Incorrect

5.3 What season are we in?
Season
☐[1] Correct
☐[2] Incorrect

5.4 What is your age?
☐[1] Correct
☐[2] Incorrect

5.5 What is your telephone number (including code)?
Code + number
☐[1] Correct
☐[2] Incorrect

Registration/Free recall
5.6 I'm going to read you a list of 10 words. Please listen carefully and try to remember them. When I am done, tell me as many as you can in any order. Ready?

(Read words from list below).

Now, tell me all the words you can remember.
Yes No
☐1 ☐2 Cabin
☐1 ☐2 Pipe
☐1 ☐2 Elephant
☐1 ☐2 Chest
☐1 ☐2 Silk
☐1 ☐2 Theatre
☐1 ☐2 Watch
☐1 ☐2 Whip
☐1 ☐2 Pillow
☐1 ☐2 Giant

Attention/Calculation
5.7 Please take 7 away from 100
Answer: 93
☐1 Correct
☐2 Incorrect

Now continue to take 7 away from what you have left over until I ask you to stop.
Answer: 86
☐1 Correct
☐2 Incorrect

Answer: 79
☐1 Correct
☐2 Incorrect

Answer: 72
☐1 Correct
☐2 Incorrect

Answer: 65
☐1 Correct
☐2 Incorrect

5.8 Please count backwards from 20 to 1.
No mistakes?
☐1 Yes
☐2 No

Comprehension, semantic & recent memory
5.9 What do people usually use to cut paper?
Answer: Scissors
☐[1] Correct
☐[2] Incorrect

5.10 What is the prickly green plant found in the desert?
Answer: Cactus
☐[1] Correct
☐[2] Incorrect

5.11 Who is the reigning monarch?
Answer: Elizabeth, Queen Elizabeth, Queen Elizabeth the 2nd
☐[1] Correct
☐[2] Incorrect

5.12 Who is the Prime Minister now?
Answer: Gordon Brown (if changed write in here_____)
☐[1] Correct
☐[2] Incorrect

5.13 What is the opposite of East?
Answer: West
☐[1] Correct
☐[2] Incorrect

Language/repetition
5.14 Please say this, 'Methodist Episcopal'.
Was this pronounced exactly right?
☐[1] Yes
☐[2] No

5.15 Please repeat the list of 10 words I read earlier.
Yes No
☐[1] ☐[2] Cabin
☐[1] ☐[2] Pipe
☐[1] ☐[2] Elephant
☐[1] ☐[2] Chest
☐[1] ☐[2] Silk
☐[1] ☐[2] Theatre
☐[1] ☐[2] Watch
☐[1] ☐[2] Whip
☐[1] ☐[2] Pillow
☐[1] ☐[2] Giant

NART

Interviewer:

I want you to read slowly down this list of words starting here. *(Hand patient NART word list and indicate CHORD).* After each word please wait until I say 'next' before reading the next word. I must warn you that there are many words that you probably won't recognise; in fact most people don't know them, so just have a guess at these, O.K.? Go ahead:

Column 1

5.17 CHORD (körd)
☐[1] Correct
☐[2] Incorrect

5.18 ACHE (āk)
☐[1] Correct
☐[2] Incorrect

5.19 DEPOT (dep'ō)
☐[1] Correct
☐[2] Incorrect

5.20 AISLE (īl)
☐[1] Correct
☐[2] Incorrect

5.21 BOUQUET (bōōk'ā, bōōkā', bōkā')
☐[1] Correct
☐[2] Incorrect

5.22 PSALM (säm)
☐[1] Correct
☐[2] Incorrect

5.23 CAPON (kā'pn)
☐[1] Correct
☐[2] Incorrect

5.24 DENY (di-nī)
☐[1] Correct
☐[2] Incorrect

5.25 NAUSEA (nö'si-ə, nö'zhə)
☐[1] Correct
☐[2] Incorrect

5.26 DEBT (det)
☐[1] Correct
☐[2] Incorrect

5.27 COURTEOUS (kûrt'yəs)
☐1 Correct
☐2 Incorrect

5.28 RAREFY (rār'i-fī)
☐1 Correct
☐2 Incorrect

5.29 EQUIVOCAL (i-kwiv'ə-kl)
☐1 Correct
☐2 Incorrect

5.30 NAÏVE (nä-ēv)
☐1 Correct
☐2 Incorrect

5.31 CATACOMB (kat'ə'kōōm)
☐1 Correct
☐2 Incorrect

5.32 GAOLED (jāld)
☐1 Correct
☐2 Incorrect

5.33 THYME (tīm)
☐1 Correct
☐2 Incorrect

5.34 HEIR (àr)
☐1 Correct
☐2 Incorrect

5.35 RADIX (rā'diks)
☐1 Correct
☐2 Incorrect

5.36 ASSIGNATE (as'-ig-nāt)
☐1 Correct
☐2 Incorrect

5.37 HIATUS (hī-ā'təs)
☐1 Correct
☐2 Incorrect

5.38 SUBTLE (sut'l)
☐1 Correct
☐2 Incorrect

5.39 PROCREATE (prō'kri-àt)
☐[1] Correct
☐[2] Incorrect

5.40 GIST (jist)
☐[1] Correct
☐[2] Incorrect

5.41 GOUGE (gowj)
☐[1] Correct
☐[2] Incorrect

Column 2
5.42 SUPERFLUOUS (sōō-pûr'flŏŏ-əs, sū-pûr'flŏŏ-əs)
☐[1] Correct
☐[2] Incorrect

5.43 SIMILE (sim'i-li)
☐[1] Correct
☐[2] Incorrect

5.44 BANAL (bən-al')
☐[1] Correct
☐[2] Incorrect

5.45 QUADRUPED (kwod'rŏŏ-ped)
☐[1] Correct
☐[2] Incorrect

5.46 CELLIST (chel'ist)
☐[1] Correct
☐[2] Incorrect

5.47 FAÇADE (fa-säd')
☐[1] Correct
☐[2] Incorrect

5.48 ZEALOT (zel'ət)
☐[1] Correct
☐[2] Incorrect

5.49 DRACHM (dram)
☐[1] Correct
☐[2] Incorrect

5.50 AEON (ē'on)
☐[1] Correct
☐[2] Incorrect

5.51 PLACEBO (plə-sē'bō)
☐[1] Correct
☐[2] Incorrect

5.52 ABSTEMIOUS (ab-stē'mi-əs)
☐[1] Correct
☐[2] Incorrect

5.53 DÉTENTE (dā-tát)
☐[1] Correct
☐[2] Incorrect

5.54 IDYLL (id'il, id'əl)
☐[1] Correct
☐[2] Incorrect

5.55 PUERPERAL (pū-ûr'pər-əl)
☐[1] Correct
☐[2] Incorrect

5.56 AVER (ə-vûr')
☐[1] Correct
☐[2] Incorrect

5.57 GAUCHE (gō sh)
☐[1] Correct
☐[2] Incorrect

5.58 TOPIARY (tō'pi-ə-ri)
☐[1] Correct
☐[2] Incorrect

5.59 LEVIATHIAN (le-vi'ə-thən)
☐[1] Correct
☐[2] Incorrect

5.60 BEATIFY (bi-at'i-fi)
☐[1] Correct
☐[2] Incorrect

5.61 PRELATE (prel'it)
☐[1] Correct
☐[2] Incorrect

5.62 SIDEREAL (sī-dē'ri-əl)
☐[1] Correct
☐[2] Incorrect

5.63 DEMESNE (di-mān, di-mēn)
☐[1] Correct
☐[2] Incorrect

5.64 SYNCOPE (sing'kə-pē)
☐[1] Correct
☐[2] Incorrect

5.65 LABILE (lā'bil)
☐[1] Correct
☐[2] Incorrect

5.66 CAMPANILE (kam-pan-ē'lā, kam-pan-ē'lē)
☐[1] Correct
☐[2] Incorrect

6. Medication Review / Current Treatment

6.1 Herbal Remedies
☐[1] Yes
☐[2] No

Name	Total daily dose	Units
a.		
b.		
c.		
d.		
e.		
f.		

6.2 Diabetes tablets
☐[1] Yes
☐[2] No

Name	Total daily dose	Units
a. Metformin (Glucophage)		
b. Acarbose		
c. Repaglinide (Prandin)		
d. Nateglinide (Starlix)		
e. Glibenclamide (Daomil, Euglucon)		
f. Gliclazide (Diamicron)		
g. Glimepiride (Amaryl)		
h. Glipizide (Glibenese, Minodiab)		
i. Other *Name:*		

6.3 Insulin
☐[1] Yes
☐[2] No

Name	Dose (Units)	Frequency
a. Insulin Lispro (Humalog)		☐ Once daily ☐ Twice daily ☐ Three times daily ☐ Other:
b. Insulin Aspart (NovoRapid)		☐ Once daily ☐ Twice daily ☐ Three times daily ☐ Other:
c. Insulin Glulisine (Apidra)		☐ Once daily ☐ Twice daily ☐ Three times daily ☐ Other:
d. Insulin Glargine (Lantus)		☐ Once daily ☐ Twice daily ☐ Three times daily ☐ Other:
e. Isophane Insulin (NPH) (eg. Insulatard, Humulin I)		☐ Once daily ☐ Twice daily ☐ Three times daily ☐ Other:
f. Soluble Insulin (eg. Humulin S, Actrapid)		☐ Once daily ☐ Twice daily ☐ Three times daily ☐ Other:
g. Insulin Detemir (Levemir)		☐ Once daily ☐ Twice daily ☐ Three times daily ☐ Other:
h. Humalog Mix 25		☐ Once daily ☐ Twice daily ☐ Three times daily ☐ Other:
i. Humalog Mix 50		☐ Once daily ☐ Twice daily ☐ Three times daily ☐ Other:
j. NovoMix 30 (eg. Mixtard 30)		☐ Once daily ☐ Twice daily ☐ Three times daily ☐ Other:
k. Other *Name:*		☐ Once daily ☐ Twice daily ☐ Three times daily ☐ Other:

6.4 Cholesterol – lowering medications
☐[1] Yes
☐[2] No

Name	Total daily dose	Units
a. Atvorvastatin (Lipitor)		
b. Simvastatin (Zocor)		
c. Bezafibrate (Bezalip)		
d. Fenofibrate (Lipantil)		
e. Colestyramine (Questran)		
f. Ezetumibe		
g. Other *Name:*		

6.5 Anti-hypertensives
☐[1] Yes
☐[2] No

Name	Total daily dose	Units
a. Amlodopine		
b. Ramipril (Lopace)		
c. Doxazosin		
d. Felodipine		
e. Labetalol		
f. Atenolol		
g. Other *Name:*		

6.6 Diuretics
☐[1] Yes
☐[2] No

Name	Total daily dose	Units
a. Bendroflumethiazide		
b. Furosemide		
c. Spirinolactone		
d. Hydrochlorathiazide		
e. Other *Name:*		

6.7 NSAIDS and Opiods
☐[1] Yes
☐[2] No

Name	Total daily dose	Units
a. Aspirin		
b. Ibruprofen		
c. Codeine		
d. Hydromorphone (Dilaudid)		
e. Meperidine		
f. Oxycodone		
g. Other *Name:*		

6.8 Other medications

☐[1] Yes

☐[2] No

Name	Total daily dose	Units
a.		
b.		
c.		
d.		
e.		
f.		
g.		
h.		
i.		
j.		
k.		

7. SOUL-D Lab Tests

Date obtained: ___ ___/ ___ ___ (mm/yy)

Lab: ☐[1] KCH ☐[2] GSTT ☐[3] PRU ☐[4] UHL ☐[5] QMS ☐[6]Mayday ☐[7] Other……..

Test	Units	Value	Ref. range
Lipids			
a. Triglycerides	mmol/L		0.5-2.0
b. LDL	mmol/L		1.0-3.0
c. HDL	mmol/L		>1.0
d. Total cholesterol	mmol/L		1.0-5.0
LFTs			
e. ALT (alanine aminotransferase)	IU/L		5-55
f. AST (aspartate aminotransferase)	IU/L		10-50
g. ALP (alkaline phosphatase)	IU/L		30-130
h. GGT (gamma-glutamyl transferase)	IU/L		1-55
Renal			
i. Creatinine	umol/L		45-120
j. eGFR	ml/min		
FBC			
k. Total white count (WBC)	10\S\9/l		4.00-11.00
l. Haemoglobin (Hb)	g/dl		11.5-15.5
m. Platelet count (PLT)	10\S\9/l		150-450
n. Neutrophils	10\S\9/l		0.20-6.30
o. Lymphocytes	10\S\9/l		1.30-4.00
TFTs			
p. Thyroid stimulating hormone (TSH)	mU/L		0.30-5.50
q. Free thyroxine	pmol/L		9.0-25.0
r. HbA1c	%		<7.5%
s. Prolactin	mU/L		< 410
t. C-reactive Protein	mg/l		< 5.0
u. Cortisol	nmol/L		130-580
v. Insulin levels	mU/L		4.4-26.0
w. Plasma glucose (fasting)	mmol/L		3.0-6.0
x. HOMA-IR (v x u)/22.5			
y. ACR	µg/mg		<3.0

Patient Health Questionnaire

1 Over the last two weeks, how often/how much have you been bothered by any of the following problems?

		Not at all	Several days	More than half the days	Nearly every day
a)	Little interest or pleasure in doing things	☐0	☐1	☐2	☐3
b)	Feeling down, depressed, or hopeless	☐0	☐1	☐2	☐3
c)	Trouble falling or staying asleep, or sleeping too much	☐0	☐1	☐2	☐3
d)	Feeling tired or having little energy	☐0	☐1	☐2	☐3
e)	Poor appetite or overeating	☐0	☐1	☐2	☐3
f)	Feeling bad about yourself or that you are a failure or have let yourself or your family down	☐0	☐1	☐2	☐3
g)	Trouble concentrating on things, such as reading the newspaper or watching television	☐0	☐1	☐2	☐3
h)	Moving or speaking so slowly that other people could have noticed or being so fidgety or restless that you have been moving around a lot more than usual	☐0	☐1	☐2	☐3
i)	Thoughts that you would be better off dead or of hurting yourself in some way	☐0	☐1	☐2	☐3

Stressful life events

55 In the last 12 months, have you ever lived with someone as a couple and that relationship ended in separation or divorce?

No ☐1 Yes ☐2

56 In the last 12 months, has a spouse/partner, child, or other loved one died?

No ☐1 Yes ☐2

57 In the last 12 months, have you ever seen something violent happen to someone (e.g., attacked or beaten) or seen someone killed?

No ☐1 Yes ☐2

58 In the last 12 months, have you ever been in the immediate area or place where a major natural disaster occurred such as a major fire, flood, tornado, earthquake, or other natural disaster?

No ☐1 Yes ☐2

59 In the last 12 months, have you ever had a serious accident?

No ☐1 Yes ☐2

60 In the last 12 months, has one of you children ever had a near-fatal accident or life-threatening illness?

No ☐1 Yes ☐2

61 In the last 12 months, has one of you children ever had a serious illness or disability?

No ☐1 Yes ☐2

62 In the last 12 months, have you ever been in combat in a war, lived near a war zone, or been present during a political uprising?

No ☐1 Yes ☐2

63 In the last 12 months, have you ever experienced a period where you slept in a park, abandoned building, the street, a train or bus, in a shelter for homeless people or in another temporary residence or with a friend or relative because you had no money to pay for rent?

No ☐1 Yes ☐2

64 In the last 12 months, have you ever been attacked, mugged, robbed, or been the victim of a serious crime?

No ☐1 Yes ☐2

65 In the last 12 months, has anyone ever injured you with a weapon – gun, knife, stick, etc.?

No ☐[1] Yes ☐[2]

66 In the last 12 months, has anyone ever hit you, bit you, slapped you, kicked you, or forced you to have sex against your wishes?

No ☐[1] Yes ☐[2]